J. Inst. Brew., September–October, 1993, Vol. 99, *pp.* 381–384

DETERMINATION OF ETHANOL IN BEER BY GAS CHROMATOGRAPHY (DIRECT INJECTION)—COLLABORATIVE TRIAL

By G. K. Buckee and A. P. Mundy

(Submitted on behalf of the Institute of Brewing Analysis Committee)

A method employing gas chromatography for the determination of ethanol in beer has been collaboratively tested by the Analysis Committee of the Institute of Brewing. It was judged that precision values were independent of concentration over the range 0.93 to 6.05% V/V ethanol. Repeatability (r_{95}) and reproducibility (R_{95}) values of 0.061 and 0.136 respectively, were obtained over this range. At a mean level of 9.17% V/V, the r_{95} and R_{95} values were 0.154 and 0.284 respectively. This was probably due to dilution errors as the sample had to be diluted to bring it within the linear range of the method. A comparison of the precision values given by the gas chromatographic method, with those obtained in 1991/1992 by 8 laboratories in a major brewing company using 12 sample pairs, for the IOB Recommended Distillation Method, revealed that there is no significant difference between the precision data for the two methods.

Key Words: *Alcohol, beer (analysis method for), collaborative test, ethanol, gas chromatography.*

The Analysis Committee appointed the following sub-committee to determine the precision of a gas chromatographic method for the estimation of ethanol in beer: G. K. Buckee (Chairman), E. Collins, B. Johnson, A. L. Macpherson, K. McIlroy, A. P. Mundy and I. H. L. Ormrod.

Introduction

The current reference method[1] recommended by the Insitute of Brewing for determining alcohol in beer relies on distillation followed by a specific gravity measurement on the distillate. However, this procedure is labour intensive, cannot be readily automated and requires the operator to have analytical skills. Therefore many laboratories have turned to alternative methods which are rapid and have the capability of automation. One such alternative is a method relying on gas chromatography and direct injection of the sample. In contrast to the gas chromatographic procedure, the distillation method is not specific for ethanol, since it is subject to interference from other such materials. As an approximation, the contribution from the volatile constituents is in the region of 0.02% V/V. An additional advantage of the gas chromatographic method is that it is amenable to traceability to National Standards, e.g. certified ethanol solutions are now available from the Laboratory of the Government Chemist.

In view of the proposed change in duty payment in the United Kingdom to a system based on the alcoholic content of the product, the Analysis Committee decided to collaboratively test a gas chromatographic method incorporating direct injection of the sample.

Experimental

The organization of the collaborative trial and the statistical treatment of the data were carried out according to the procedures given in the International Standard ISO 5725[2]. A uniform design was employed and six samples of beer, covering the approximate range 1 to 9% V/V ethanol, were distributed to eleven laboratories, which included six from one major brewing company. In addition, an ethanol solution (5.160% V/V) was provided for calibration and a duplicate standard was included which was treated as an unknown sample. Participants were requested to determine the ethanol content of the samples in duplicate to three places of decimals, using the gas chromatographic method, details of which are given in the Appendix.

One laboratory reported dissatisfaction with their results, since extreme tailing of peaks was observed, which led to poor resolution. For this reason, their results were excluded from the calculation of precision.

Results and Discussion

Raw data as received are presented in Table 1. Two outliers were identified for the beer samples and one for the aqueous ethanol solution ($p \leq 0.01$). A summary of the results of a statistical treatment of the data is shown in Table II. Both repeatability (r_{95}) and reproducibility (R_{95}) values were judged to be independent over the range of 0.93 to 6.05% V/V. Values of r_{95} and R_{95} over this range were 0.061 and 0.136 respectively. Whilst at a mean level of 9.17% V/V, the values were 0.154 and 0.284 respectively. The poorer precision at the 9% V/V level is probably due to dilution errors, as this sample had to be diluted in the ratio of 1:1 to bring it within the linear range of the method. The precision values obtained by the gas chromatographic method compare favourably with those given for the distillation method in the IOB Recommended Methods of Analysis, viz. r_{95}, 0.076, and R_{95}, 0.182, over the range 2.64 to 4.56% V/V. However, since these values were produced between 1981 and 1984, by 16 laboratories in a single major brewing company using 34 sample pairs, it is perhaps more appropriate to make the comparison with values of r_{95}, 0.081, and R_{95}, 0.138, which were produced by 8 laboratories in the same company, using 12 sample pairs in 1991/1992. Using these latter values, it seems that there is no significant difference between the precision of the two methods.

Conclusions

There is no significant difference between the precision data obtained by the gas chromatographic method and the IOB Recommended Distillation Method. The Analysis Committee of the IOB judged that the precision values obtained in the collaborative trial were acceptable and approved the inclusion in Recommended Methods of the gas chromatographic procedure for determining ethanol in beer.

References

1. Institute of Brewing, *Recommended Methods of Analysis*, 1991, Method 8.5.3, p. 220.
2. *Precision of test methods—Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*, ISO 5725, 1986.

TABLE I. Sample results as received

	Ethanol (% V/V)													
	Beer samples												Ethanol sample	
Laboratory No.	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2	G1	G2
1	0.920	0.890	3.390	3.410	4.430	4.430	4.840	4.850	6.120	6.120	9.240	9.240	5.130	5.160
2	1.027	0.995	3.402	3.442	4.351	4.403	4.724	4.730	5.943	5.951	9.072	8.990	5.122	5.129
3	0.840	0.900	3.410	3.440	4.500	4.430	4.850	4.850	6.120	6.070	9.080	8.990	5.170	5.180
4	0.938	0.932	3.467	3.434	4.463	4.457	4.871	4.873	6.074	6.080	9.129	9.236	5.193	5.190
5	0.863	0.851	3.143*	3.390*	4.375	4.444	4.800	4.819	6.057	6.051	9.143	9.320	5.130	5.117
6	0.971	0.922	3.401	3.423	4.368	4.422	4.817	4.820	5.996	6.048	9.092	9.110	5.181	5.169
7	0.951	0.953	3.505	3.487	4.451	4.472	4.889	4.840	6.058	6.082	9.154	9.218	5.164	5.128
8	0.943	0.934	3.467	3.465	4.501	4.481	4.881	4.899	6.112	6.058	–	–	5.166	5.171
9	0.979	0.961	3.460	3.469	4.442	4.461	4.861	4.868	6.007	6.038	9.293	9.245	5.127	5.143
10	0.917	0.908	3.542*	3.396*	4.374	4.394	4.776	4.712	5.998	5.945	9.243	9.228	4.961*	5.171*
11†	0.991	0.775	3.159	3.360	3.657	3.886	4.468	4.593	5.839	5.945	7.689	8.257	4.988	5.190

*Rejected as outlier $p \leq 0.01$
†results omitted from calculation of precision

TABLE II. Summary of precision data (concentations in % V/V)

Beer	No. of laboratories	Mean m	Repeatability		Reproducibility	
			r_{95}	S_r	R_{95}	S_R
A	10	0.93	0.050	0.018	0.133	0.048
B	8	3.44	0.049	0.018	0.099	0.035
C	10	4.43	0.082	0.029	0.125	0.045
D	10	4.83	0.054	0.019	0.160	0.057
E	10	6.05	0.071	0.025	0.164	0.059
F*	9	9.17	0.154	0.055	0.284	0.101
G†	9	5.15	0.036	0.013	0.074	0.026

*Sample diluted 1:1 prior to analysis
†Aqueous ethanol solution

APPENDIX

The Determination of Ethanol in Beer by Gas Chromatography

Warning and Safety Precautions

For the recommended practices for the safe storage and handling of compressed gases, refer to the BOC Users' Guide. Both ethanol and n-butanol are flammable and must be kept away from sources of ignition. n-Butanol is harmful by inhalation and also by adsorption through skin.

1 SCOPE

The determination of ethanol in beer by gas liquid chromatography using direct injection.

2 FIELD OF APPLICATION

2.1 The method can be used to determine the ethanol content of all beers and partly-fermented worts, but not non-alcoholic beers, i.e. with ethanol contents below 0.5% V/V. The method can be used to determine the alcohol content of shandy. The method is applicable to products with an ethanol concentration between 0.5 and 8% V.V.

3 PRINCIPLE

3.1 The beer sample is de-gassed by filtration and attemperated to 20°C.

3.2 The sample is diluted with a known quantity of n-butanol (as an internal standard).

3.3 The diluted liquid sample is injected into a gas chromatographic column, previously calibrated using standard ethanol solutions.

3.4 The percentage of ethanol V/V in the sample is calculated from the peak areas of the ethanol and n-butanol peaks, using a previously calculated calibration factor.

4 REAGENTS

4.1 Unless otherwise stated, use only reagents of recognized analytical grade and distilled water, or water of equivalent purity.

4.2 Ethanol, absolute. Lower strengths may be used provided the concentration is known.

4.3 n-Butanol, new batches must be checked chromatographically to ensure that there are no interferences with the ethanol peak.

4.4 n-Butanol (0.5% V/V), internal standard. Pipette 10 ml of n-butanol into a 2 litre volumetric flask and dilute to 2 litres with distilled water.

5 APPARATUS

5.1 Gas chromatograph, fitted with a flame ionization detector and an oven capable of operating isothermally at 115°C.

5.2 Auto-sampler for the gas chromatograph—to inject 0.5 or 1 μl of liquid sample, OR a 10 μl gas-tight positive displacement syringe.

5.3 Auto-diluter, capable of diluting to 10% ± 0.1%, OR 2 ml grade A bulb pipette, 20 ml grade A bulb pipette.

5.4 Computing integrator, OR Chart recorder and integrator.

Care must be taken when interpreting computed signals from integrators; they should not be assumed to be accurate. If possible, the peak areas given should be checked against a manual calculation made by measuring peak height and width. For this purpose, a trace of peaks should always be produced in addition to the printed value for peak area.

5.5 Water bath, controlled to 20°C ± 0.5°C.

5.6 Thermometer—accurate to ± 0.1°C.

5.7 Chromatography column 2 metres × 2 mm, glass or stainless steel packed with 15% Carbowax 20M on Chromosorb WAW DMCS, 100/100 mesh (e.g. Phase Separations).

5.8 Filter funnels.

5.9 Filter paper—Watman No. 1.

5.10 100 ml conical flask.

5.11 Ultrasonic bath.

5.12 Pipettes—Grade A bulk 1 ml, 5 ml, 3 ml and 20 ml.

5.13 Volumetric flasks—Grade A, 250 ml and 100 ml.

6 *SAMPLE PREPARATION*

6.1 Adjust the temperature of the sample to 20°C and filter 50 ml into a conical flask. Discard the first 25 ml of filtrate and cover the filter funnel with a clock glass to prevent loss of ethanol.

6.2 Place the flask in the ultrasonic bath and sonicate to remove any remaining carbon dioxide. Seal the flask until required for analysis.

7 *PROCEDURE*

7.1 Preparation of Standards

7.1.1 Ethanol standard 1% V/V, accurately pipette 1.0 ml of ethanol into a 100 ml volumetric flask. Make up to volume with distilled water at 20.0°C ± 0.1°C.

7.1.2 Ethanol standard 5% V.V, prepare as in 7.1.1 using 5.0 ml of ethanol.

7.1.3 Ethanol standard 8% V/V, accurately pipette 20.0 ml of ethanol into a 250 ml volumetric flask. Make up to volume with water at 20.0°C ± 0.1°C.

7.1.4 Ensure that pipettes, syringes, ethanol, water and resulting diluted solutions are all at 20.0°C ± 0.1°C.

7.1.5 Check the ethanol content of the standard solutions by measuring the specific gravities either by gravity bottle or meter.

7.2 Calibration

7.2.1 Attemperate the n-butanol internal standard and the ethanol calibration standards to 20°C ± 0.1°C in the water bath.

7.2.2 Prepare the gas chromatograph according to the manufacturer's instructions.
Typical conditions:

Oven temperature	115°C
Injector temperature	150°C
Detector temperature	200°C
Carrier gas	Nitrogen
Carrier gas flow rate	45 ml/min

7.2.3 Dilute 2 ml of each ethanol standard with 20 ml of n-butanol internal standard using an auto-diluter or by pipetting, into a clean, dry 50 ml conical flask.

Note: Ensure that both solutions are at 20°C ± 0.1°C before diluting. Temperature accuracy at this stage is critical to the accuracy of the method. Mix thoroughly.

7.2.4 Inject 0.5 or 1 μl of the first diluted standard ethanol solution from 7.2.3 into the gas chromatograph.

7.2.5 Determine the areas of the ethanol and n-butanol internal standard peaks.

7.2.6 Repeat steps 7.2.4 and 7.2.5 for each standard ethanol solution in duplicate.

7.3 Analysis

7.3.1 Attemperate the filtered, degassed beer sample to 20°C ± 0.1°C.

7.3.2 Dilute 2 ml of the beer sample with 20 ml of n-butanol internal standard using the same equipment as for the ethanol standards, into a clean, dry 50 ml conical flask. Mix thoroughly.

7.3.3 Inject 0.5 or 1 μl of the dilute beer from 7.3.2 into the gas chromatograph.

7.3.4 Calculate the area of the peaks due to the ethanol and n-butanol internal standard.

8 *CALCULATION*

8.1 Plot a graph of

$$\frac{\text{Area of ethanol peak}}{\text{Area of internal standard peak}}$$

against ethanol concentration V/V (after correcting for purity) from the results obtained for each of the calibration standards. The graph should be linear and should pass through the origin.

8.2 Fit either visually or by using linear regression, the best straight line to the graph. Calculate the coefficient of correlation (r) and provided $r > 0.99$, calculate the gradient to give the factor F.

$$F = \frac{\text{Ethanol concentration}}{\text{Area ethanol peak/Area internal standard peak}}$$

8.3 Once the linearity of the calibration range is established, subsequent calibrations to determine the factor F can be performed by carrying out triplicate determinations of the standard nearest to the expected concentration of ethanol in the sample.

8.4 From the results obtained for the sample, calculate:

$$\frac{\text{Area of ethanol peak}}{\text{Area of internal standard peak}}$$

Either read off the corresponding concentration of ethanol from the standard curve, or calculate the % ethanol V/V by multiplying by the factor F.

8.5 Express the results as ethanol % V/V at 20°C, to two decimal places.