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# Determination of the accuracy, repeatability and reproducibility of a method for the determination of alcohol in beer by Near Infra Red Spectroscopy

Submitted on behalf of the Analysis Committee of the European Brewery Convention.

A method for the determination of alcohol in beer by Near Infra Red Spectroscopy was collaboratively tested by the Analysis Committee of the European Brewery Convention according to ISO Standard 5725 in order to determine its suitability for publication in *Analytica-EBC*. Accuracy, repeatability  $r_{95}$  and reproducibility  $R_{95}$  values are presented.

The NIR method was found to have better within and between laboratory precision than the reference distillation method for the determination of alcohol.

Repeatability  $r_{95}$  was found to be independent of the mean value and was 0,032 % V/V over the range 2,18 to 8,77 % V/V alcohol. Reproducibility  $R_{95}$  was found to be dependent on the mean and is  $0,017m + 0,006$  % V/V over the range of alcohol tested. The NIR method has acceptable accuracy when compared to the distillation method.

The method is recommended for inclusion in *Analytica-EBC*.

Descriptors: *Analytica-EBC*, method development, determination of alcohol, NIR-spectroscopy

## 1 Introduction

The Analysis Committee of the European Brewery Convention decided to carry out an interlaboratory collaborative trial to determine the accuracy and precision of a method for the determination of alcohol in beer by Near Infra Red Spectroscopy (NIR).

## 2 Experimental

The organisation of the trial and the statistical treatment of the data was carried out according to the procedure given in the International Standard ISO 5725-2.

Duplicate samples of five commercially produced and packaged beers, each from a single homogeneous batch, covering the approximate alcohol range 2,0 – 9,0 % v/v were sent to 24 participating laboratories.

Participants were asked to determine the alcohol content of each sample by NIR using the draft EBC method (appended), using their own choice of NIR instrument. Participants were asked to complete a short questionnaire that requested details of the type of instrument used (make and model) and their calibration procedure.

Four sets of the same samples were sent to a further four “reference” laboratories for analysis of alcohol by the distillation procedure so that the accuracy of the NIR method could be determined.

## 3 Results and discussion

The basic data received from the participating laboratories are shown in Table 1.

### 3.1 Analysis of data

The statistical evaluation included the following steps:

- 1 Analysis of the homogeneity of the within laboratory variance
- 2 Analysis of outliers among the within laboratory means.
- 3 Calculation of repeatability  $r_{95}$  and reproducibility  $R_{95}$
- 4 Determination of the dependence of the precision on the mean

### 3.2 Outliers and stragglers – see Table 2

Grubb’s test detected an outlier for sample A – this laboratory’s result was rejected from the calculation of the precision data for sample A.

Cochran’s test detected two outliers for sample B and both these were rejected from the overall calculation of precision.

One laboratory was detected as an outlier for Sample E by Cochran’s test and two further outliers were detected for this sample by Grubb’s test – all these 3 laboratories were rejected as outliers for sample E.

Grubbs test detected a straggler for sample D, but the results from this laboratory were retained.

### 3.3 Precision

The results for the calculation of precision data are shown in Table 3.

Repeatability  $r_{95}$  was found to be independent of the mean value

Participant	Instrument used	Water check	Calibration	Beer check
1	Paar Alcoalyzer			
2	Foss Infratec 1256		0–11 %	
3	Foss Infratec 1256		0–11 %	
4	Foss Infratec 1256		0–11 %	
5	Foss Infratec 1256		0–11 %	
6	Paar Alcoalyzer	daily	daily 10 % v/v	daily 5,4 % v/v
7	Paar Alcoalyzer	daily	weekly 9,6 % v/v	no
8	Paar Alcoalyzer			
9	Paar Alcoalyzer	yes	daily 5 % v/v	yes 5,2 % vol
10	Paar Alcoalyzer	daily	daily 11 % v/v	daily 4,98 % v/v
11	Paar Alcoalyzer	yes	yes 10 % v/v	yes 4,97 % v/v
12	Paar Alcoalyzer	daily	daily 0–10 % v/v	
13	Paar Alcoalyzer	yes	yes 8,61 % v/v	yes 4,46 % v/v
14	Paar Alcoalyzer	yes	yes 9,61 % v/v	yes 5,37 % v/v
15	Paar Alcoalyzer	daily	daily 4,93 % v/v	no
16	Paar Alcoalyzer	daily	weekly 10–12 % v/v	no
17	Foss Rapitec	no	no	yes, 1 in every 10 samples
18	Paar Alcoalyzer	daily	daily 10,05 % v/v	no
19	Paar Alcoalyzer	daily	daily 10 % v/v	no
20	Paar Alcoalyzer DMA 4500	daily	daily 8,7 % v/v	
21	Paar Alcoalyzer 560771	daily	monthly 10 % v/v	monthly
22	Paar Alcoalyzer plus	yes	yes 9,64 % v/v	yes 4,5 % v/v
23	Paar Alcoalyzer	daily	alcohol 0,45 % vol, alcohol 12 % vol	yes, daily
24	Foss Infratec 1256	yes	yes	yes 5 %

and equal to 0,032 % V/V.

Reproducibility  $R_{95}$  was found to be dependent on the mean and is given by  $0,017m + 0,006$  % V/V.

There were insufficient laboratories using the Foss equipment to enable a robust statistical evaluation of the comparative precision between the Foss Infratec and Paar Alcoalyzer instruments. Tables 4 and 5 give the raw data and precision calculations for the laboratories using the Paar Alcoalyzer and the Foss instruments, respectively. The precision data from the two instrument types indicate that there is no significant difference in their accuracy or precision. However, the one laboratory using the Foss Rapitec was identified as an outlier for 2 out of the 5 samples.

A comparison of the NIR results to the results from the distillation method show that the difference between the distillation and the NIR results for each sample are within the range of reproducibility of the distillation method. It is therefore concluded that the NIR method has acceptable accuracy when compared to the distillation method (which is the accepted reference procedure). A comparison of the grand mean value from each level suggests an alcohol recovery by the NIR method of 99,1 %.

#### 4 Questionnaire results

The Paar Alcoalyzer was the most popular instrument used in the test.

The procedure for checking and calibrating the instrument varied between participants; most carried out a water zero check on a daily basis and a daily calibration check with a standard ethanol solution, although the concentration of the standard used varied considerably. A majority of participants also carried out a calibration check using a beer sample on a daily basis.

The method recommends a daily or weekly check with water and an ethanol standard solution and a standard beer sample (see above).

#### 5 Conclusion

The NIR method was found to have better within and between laboratory precision than the reference distillation method for the determination of alcohol.

Repeatability  $r_{95}$  was found to be independent of the mean value and was 0,032 % V/V over the range 2,18 to 8,76 %V/V alcohol.

Reproducibility  $R_{95}$  was found to be dependent on the mean and is  $0,017m + 0,006$  % V/V over the range of alcohol tested.

The NIR method has acceptable accuracy when compared to the distillation method.

The method is recommended for inclusion in Analytica-EBC.

## Appendix

Table 1 Basic Data Results abv % v/v

Participant ID No.	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	Instrument used
Ref 1	2,17	2,17	4,88	4,87	3,73	3,73	7,46	7,48	8,74	8,72	Distillation
Ref 2	2,14		4,902		3,728		7,392		8,582		Distillation
Ref 3	2,166		4,906		3,767		7,494		8,735		Distillation
Ref 4	2,144	2,165	4,801	4,801	3,672	3,736	7,288	7,276	8,538	8,562	Distillation
1	2,15	2,16	4,9	4,89	3,74	3,74	7,5	7,48	8,63	8,78	Not provided
2	2,21	2,2	4,9	4,9	3,73	3,74	7,48	7,47	8,83	8,84	Foss Infratec 1256
3	2,18	2,18	4,89	4,89	3,71	3,72	7,48	7,47	8,85	8,86	Foss Infratec 1256
4	2,2	2,18	4,9	4,9	3,72	3,72	7,46	7,45	8,82	8,83	Foss Infratec 1256
5	2,22	2,21	4,92	4,91	3,72	3,73	7,47	7,46	8,82	8,83	Foss Infratec 1256
6	2,22	2,19	4,9	4,89	3,76	3,76	7,44	7,47	8,76	8,79	Paar Alcolyzer
7	2,21	2,2	4,91	4,92	3,77	3,77	7,48	7,48	8,78	8,78	Paar Alcolyzer
8	2,16	2,17	4,9	4,89	3,73	3,74	7,46	7,46	8,75	8,74	Paar Alcolyzer
9	2,16	2,13	4,89	4,89	3,74	3,73	7,47	7,47	8,77	8,76	Paar Alcolyzer
10	2,19	2,21	4,91	4,91	3,74	3,74	7,49	7,48	8,8	8,79	Paar Alcolyzer
11	2,14	2,15	4,85	4,86	3,71	3,71	7,42	7,43	8,7	8,73	Paar Alcolyzer
12	2,18	2,2	4,91	4,91	3,76	3,75	7,43	7,45	8,74	8,72	Paar Alcolyzer
13	2,18	2,17	4,82	4,89	3,77	3,78	7,5	7,53	9	9	Paar Alcolyzer
14	2,17	2,17	4,87	4,86	3,72	3,72	7,41	7,41	8,71	8,67	Paar Alcolyzer
15	2,219	2,174	4,959	4,935	3,752	3,742	7,627	7,572	9,06	8,989	Paar Alcolyzer
16	2,17	2,16	4,88	4,88	3,73	3,73	7,46	7,46	8,76	8,77	Paar Alcolyzer
17	2,3	2,28	4,92	4,96	3,79	3,79	7,41	7,45	8,91	8,85	Foss Rapitec
18	2,19	2,18	4,87	4,89	3,73	3,74	7,47	7,46	8,77	8,75	Paar Alcolyzer
19	2,19	2,16	4,88	4,88	3,74	3,74	7,43	7,44	8,68	8,72	Paar Alcolyzer
20	2,14	2,14	4,86	4,87	3,72	3,72	7,42	7,45	8,72	8,75	Paar Alcolyzer
21	2,17	2,18	4,91	4,91	3,78	3,78	7,39	7,41	8,67	8,62	Paar Alcolyzer
22	2,18	2,19	4,9	4,9	3,75	3,75	7,47	7,47	8,77	8,76	Paar Alcolyzer plus
23	2,18	2,18	4,9	4,9	3,75	3,74	7,48	7,48	8,78	8,79	Paar Alcolyzer
24	2,18	2,22	4,88	4,88	3,73	3,75	7,34	7,34	8,76	8,75	Foss Infratec 1256

Table 2 Results of statistical evaluation of outliers and stragglers

Lab	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2
1	2,15	2,16	4,90	4,89	3,74	3,74	7,50	7,48	8,63**	8,78**
2	2,21	2,20	4,90	4,90	3,73	3,74	7,48	7,47	8,83	8,84
3	2,18	2,18	4,89	4,89	3,71	3,72	7,48	7,47	8,85	8,86
4	2,20	2,18	4,90	4,90	3,72	3,72	7,46	7,45	8,82	8,83
5	2,22	2,21	4,92	4,91	3,72	3,73	7,47	7,46	8,82	8,83
6	2,22	2,19	4,90	4,89	3,76	3,76	7,44	7,47	8,76	8,79
7	2,21	2,20	4,91	4,92	3,77	3,77	7,48	7,48	8,78	8,78
8	2,16	2,17	4,90	4,89	3,73	3,74	7,46	7,46	8,75	8,74
9	2,16	2,13	4,89	4,89	3,74	3,73	7,47	7,47	8,77	8,76
10	2,19	2,21	4,91	4,91	3,74	3,74	7,49	7,48	8,80	8,79
11	2,14	2,15	4,85	4,86	3,71	3,71	7,42	7,43	8,70	8,73
12	2,18	2,20	4,91	4,91	3,76	3,75	7,43	7,45	8,74	8,72
13	2,18	2,17	4,82**	4,89**	3,77	3,78	7,50	7,53	9,00 <sup>oo</sup>	9,00 <sup>oo</sup>
14	2,17	2,17	4,87	4,86	3,72	3,72	7,41	7,41	8,71	8,67
15	2,22	2,17	4,96	4,94	3,75	3,74	7,63 <sup>o</sup>	7,57 <sup>o</sup>	9,06 <sup>oo</sup>	8,99 <sup>oo</sup>
16	2,17	2,16	4,88	4,88	3,73	3,73	7,46	7,46	8,76	8,77
17	2,30 <sup>oo</sup>	2,28 <sup>oo</sup>	4,92**	4,96**	3,79	3,79	7,41	7,45	8,91	8,85
18	2,19	2,18	4,87	4,89	3,73	3,74	7,47	7,46	8,77	8,75
19	2,19	2,16	4,88	4,88	3,74	3,74	7,43	7,44	8,68	8,72
20	2,14	2,14	4,86	4,87	3,72	3,72	7,42	7,45	8,72	8,75
21	2,17	2,18	4,91	4,91	3,78	3,78	7,39	7,41	8,67	8,62
22	2,18	2,19	4,90	4,90	3,75	3,75	7,47	7,47	8,77	8,76
23	2,18	2,18	4,90	4,90	3,75	3,74	7,48	7,48	8,78	8,79
24	2,18	2,22	4,88	4,88	3,73	3,75	7,34 <sup>o</sup>	7,34 <sup>o</sup>	8,76	8,75

\* detected as straggler by Cochran's test; retained  
 \*\* detected as outlier by Cochran's test; rejected  
 ° detected as straggler by Grubb's test; retained  
 °° detected as outlier by Grubb's test; rejected

**Table 3 Precision data – all participants**

	A	B	C	D	E
n	23	22	24	24	21
$s_r^2$	0,000188	0,000040	0,000029	0,000194	0,000331
$S_L^2$	0,0003	0,0004	0,0004	0,0021	0,0030
$s_r$	0,0137	0,0064	0,0054	0,0139	0,0182
$s_R$	0,0213	0,0208	0,0213	0,0476	0,0581
<b>m</b>	2,180	4,894	3,742	7,459	8,768
$r_{95}$	0,038	0,018	0,015	0,039	0,051
$R_{95}$	0,065	0,058	0,060	0,133	0,163
$CVS_r$	0,63	0,13	0,14	0,19	0,21
$CVS_R$	1,06	0,42	0,57	0,64	0,66

The repeatability limit ( $r_{95}$ ) does not depend on the mean.  
 The reproducibility limit ( $R_{95}$ ) depends on the mean:  $R_{95} = 0,025 + 0,014m$ .

**Table 4a Paar Alcohyser – raw data**

Lab	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2
6	2,22	2,19	4,90	4,89	3,76	3,76	7,44	7,47	8,76	8,79
7	2,21	2,20	4,91	4,92	3,77	3,77	7,48	7,48	8,78	8,78
8	2,16	2,17	4,90	4,89	3,73	3,74	7,46	7,46	8,75	8,74
9	2,16	2,13	4,89	4,89	3,74	3,73	7,47	7,47	8,77	8,76
10	2,19	2,21	4,91	4,91	3,74	3,74	7,49	7,48	8,80	8,79
11	2,14	2,15	4,85	4,86	3,71	3,71	7,42	7,43	8,70	8,73
12	2,18	2,20	4,91	4,91	3,76	3,75	7,43	7,45	8,74	8,72
13	2,18	2,17	4,82**	4,89**	3,77	3,78	7,50	7,53	9,00 <sup>oo</sup>	9,00 <sup>oo</sup>
14	2,17	2,17	4,87	4,86	3,72	3,72	7,41	7,41	8,71	8,67
15	2,22	2,17	4,96	4,94	3,75	3,74	7,63 <sup>oo</sup>	7,57 <sup>oo</sup>	9,06 <sup>oo</sup>	8,99 <sup>oo</sup>
16	2,17	2,16	4,88	4,88	3,73	3,73	7,46	7,46	8,76	8,77
18	2,19	2,18	4,87	4,89	3,73	3,74	7,47	7,46	8,77	8,75
19	2,19	2,16	4,88	4,88	3,74	3,74	7,43	7,44	8,68	8,72
20	2,14	2,14	4,86	4,87	3,72	3,72	7,42	7,45	8,72	8,75
21	2,17	2,18	4,91	4,91	3,78	3,78	7,39	7,41	8,67	8,62
22	2,18	2,19	4,90	4,90	3,75	3,75	7,47	7,47	8,77	8,76
23	2,18	2,18	4,90	4,90	3,75	3,74	7,48	7,48	8,78	8,79

\* detected as straggler by Cochran's test; retained  
 \*\* detected as outlier by Cochran's test; rejected  
 ° detected as straggler by Grubb's test; retained  
 °° detected as outlier by Grubb's test; rejected

**Table 4b Precision data – Paar Alcohyser**

	A	B	C	D	E
n	17	16	17	16	15
$s_r^2$	0,000186	0,000049	0,000021	0,000122	0,000327
$S_L^2$	0,0003	0,0005	0,0004	0,0008	0,0015
$s_r$	0,0136	0,0070	0,0045	0,0110	0,0181
$s_R$	0,0224	0,0235	0,0198	0,0308	0,0432
<b>m</b>	2,177	4,894	3,744	7,455	8,743
$r_{95}$	0,038	0,020	0,013	0,031	0,051
$R_{95}$	0,063	0,066	0,056	0,086	0,121
$CVS_r$	0,63	0,14	0,12	0,15	0,21
$CVS_R$	1,03	0,48	0,53	0,41	0,49

Table 5a Foss Infratec and Rapitec – raw data

Lab	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2
2	2,21	2,20	4,90	4,90	3,73	3,74	7,48	7,47	8,83	8,84
3	2,18	2,18	4,89	4,89	3,71	3,72	7,48	7,47	8,85	8,86
4	2,20	2,18	4,90	4,90	3,72	3,72	7,46	7,45	8,82	8,83
5	2,22	2,21	4,92	4,91	3,72	3,73	7,47	7,46	8,82	8,83
17	2,30°	2,28°	4,92**	4,96**	3,79°	3,79°	7,41*	7,45*	8,91*	8,85*
24	2,18	2,22	4,88	4,88	3,73	3,75	7,34	7,34	8,76	8,75

\* detected as straggler by Cochran's test; retained

\*\* detected as outlier by Cochran's test; rejected

° detected as straggler by Grubb's test; retained

Table 5b Precision data – Foss Infratec and Rapitec

	A	B	C	D	E
n	6	5	6	6	6
$s_r^2$	0,000217	0,000000	0,000058	0,000167	0,000342
$S_L^2$	0,0014	0,0001	0,0007	0,0026	0,0016
$s_r$	0,0147	0,0000	0,0076	0,0129	0,0185
$s_R$	0,0408	0,0084	0,0279	0,0526	0,0440
<b>m</b>	2,213	4,892	3,738	7,440	8,829
$r_{95}$	0,041	0,000	0,021	0,036	0,052
$R_{95}$	0,114	0,023	0,078	0,147	0,123
$CVS_r$	0,67	0,00	0,20	0,17	0,21
$CVS_R$	1,84	0,17	0,75	0,71	0,50