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Determination of repeatability and reproducibility of EBC accepted methods: V - Beer

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

Methods published in *Analytica-EBC* for the determination of Beer Alcohol (9.2.1), Real, Apparent and Original Extracts (9.4), Real Degree of Fermentation (9.5), Colour (9.6), Final Attenuation (9.7), Bitterness (9.8), Total Nitrogen (9.9.1), Free Amino Nitrogen (9.10), Total Polyphenols (9.11), Vicinal Diketones (9.24.1), Total Carbohydrate (9.26) and Fermentable Carbohydrate (9.27) have been collaboratively tested by the European Brewery Convention Analysis Committee according to the ISO standard 5725. Repeatability (r_{95}) and reproducibility (R_{95}) values are presented.

BC 30 General (analysis methods)

Descriptors: Analysis methods, beer, collaborative test, precision.

Descriptoren: Analysenmethoden, Bier, Ringanalyse, Genauigkeit).

1 Introduction

An interlaboratory test was planned in order to obtain precision data for EBC methods already published in *Analytica-EBC*.

The general procedure followed was the same as for malt and laboratory wort described in a previous paper (1), where definitions and an example are given.

2 Procedure

Two ring tests were organized with EBC Analysis Committee members according to the ISO Standard 5725-2 (2).

In the first one (1997), six beer samples A to E were distributed to 13 laboratories for determination of Alcohol, Extracts and Real Degree of Fermentation (9.2.1, 9.4, 9.5), Bitterness (9.8), Total Polyphenols (9.11) and Total Carbohydrate (9.26). One beer sample (E) was rather different from the other ones. For Original Extract and Real Degree of Fermentation, data were split into two subpopulations when searching for a relationship between precision and the measured value. Some minor deviations from the methods were reported by a few participants, but this did not affect the results significantly.

In the second one (1998), five beer samples F to J were sent to 14 laboratories for determination of Colour (9.6), Final Attenuation Limit (9.7 followed by 8.6.1 and 8.6.2), Total Nitrogen (9.9.1, Kjeldahl), Free Amino Nitrogen (9.10), Vicinal Diketones (9.24.1, Spectrophotometry) and Fermentable Carbohydrate (9.27, HPLC).

Sample H was the only dark beer, the four other samples were Pilsen type beers.

3 Results and Comments

Results are given in tables 1.1 to 1.19 and summarized in table 2.

Coefficients of variation of reproducibility (CV_R) were calculated from R_{95} in order to evaluate the relative precision of the methods.

$$CV_R = \frac{R_{95}}{2.8 m} \times 100$$

where R_{95} = reproducibility
 m = measured value

3.1 Alcohol (EBC method 9.2.1)

Real Extract (EBC method 9.4)

Apparent Extract (EBC method 9.4)

Original Extract (EBC method 9.4)

Real Degree of Fermentation (EBC method 9.5)

Most laboratories (80%) used an electronic density meter for the measurement of specific gravity, which reflects the situation in practice nowadays.

For Apparent Extract, many zero values for repeatability were reported.

For Original Extract and Real Degree of Fermentation, samples were not uniformly distributed over the whole range of values. The Analysis Committee decided to give the precision values corresponding to the distinct sample separately.

These methods are very precise, with coefficients of variation of reproducibility (CVR) smaller than 1.8%.

Alcohol is the only determination where a relationship between r or R and the measured value can be observed.

3.2 Bitterness (EBC method 9.8)

The procedure was slightly modified by 3 laboratories: manual extraction, wrist-action shaking machine, rotary shaker at 160 rpm instead of the recommended "rotary shaker at 130 rpm".

Table 1.1 Alcohol % m/m (EBC method 9.2.1)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	11	1.72	0.049	0.083
B	11	3.10	0.041	0.055
C	12	3.42	0.022	0.070
D	11	3.96	0.031	0.117
E	11	7.00	0.080	0.191
F	11	3.81	0.069	0.131

Table 1.6 Real Degree of Fermentation % (EBC method 9.5)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	10	48.9	0.50	1.16
B	11	68.1	0.31	0.56
C	11	63.6	0.19	0.59
D	10	67.2	0.17	0.50
E	10	70.8	0.24	0.57
F	9	67.8	0.14	0.81

Table 1.2 Alcohol % v/v (EBC method 9.2.1)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	11	2.20	0.062	0.105
B	11	3.94	0.052	0.071
C	12	4.37	0.026	0.088
D	11	5.05	0.041	0.148
E	11	8.95	0.103	0.243
F	11	4.85	0.087	0.168

Table 1.7 Bitterness B.U. (EBC method 9.8)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	13	13.2	0.61	1.86
B	13	17.1	0.80	2.01
C	13	23.3	0.74	2.53
D	13	20.9	0.65	3.60
E	13	27.9	0.95	3.46
F	13	36.3	1.03	7.66

Table 1.3 Real Extract % m/m (EBC method 9.4)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	11	3.72	0.013	0.064
B	11	2.99	0.014	0.046
C	11	4.06	0.034	0.077
D	10	3.99	0.013	0.071
E	12	5.97	0.025	0.099
F	11	3.75	0.019	0.093

Table 1.8 Total Polyphenols mg/l (EBC method 9.11)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	12	73	4.2	22.4
B	12	114	4.3	34.2
C	12	161	4.4	34.9
D	12	112	4.1	28.5
E	11	176	3.5	37.0
F	12	110	3.8	45.1

Table 1.4 Apparent Extract % m/m (EBC method 9.4)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	12	2.91	0.017	0.047
B	12	1.55	0.022	0.069
C	12	2.47	0.014	0.077
D	10	2.17	0.026	0.074
E	10	2.87	0.010	0.118
F	11	2.00	0.017	0.098

Table 1.9 Total Carbohydrate g/100 ml (EBC method 9.26)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	9	3.18	0.086	0.809
B	10	2.32	0.124	0.500
C	10	3.31	0.079	0.643
D	8	3.10	0.104	0.491
E	10	4.62	0.172	1.361
F	10	2.65	0.081	0.639

Table 1.5 Original Extract % m/m (EBC method 9.4)

	Number of results	Mean	r ₉₅	R ₉₅
Beer A	10	7.14	0.071	0.185
B	12	9.11	0.089	0.164
C	11	10.73	0.065	0.161
D	10	11.67	0.063	0.196
E	11	19.02	0.147	0.384
F	9	11.16	0.059	0.218

Table 1.10 Colour EBC units (EBC method 9.6)

	Number of results	Mean	r ₉₅	R ₉₅
Beer G	14	9.46	0.174	0.869
H	12	115	2.214	10.84
I	14	10.3	0.421	1.066
J	14	8.84	0.158	0.728
K	14	11.9	0.159	0.648

Table 1.11 Final Attenuation %, Reference Method (EBC methods 9.7 and 8.6.1)

	Number of results	Mean	r_{95}	R_{95}
Beer G	8	69.5	0.747	3.055
H	8	97.6	0.606	2.491
I	9	83.5	0.229	2.824
J	9	84.2	0.263	1.781
K	9	81.1	0.391	2.946

Table 1.12 Final Attenuation %, Rapid Method (EBC methods 9.7 and 8.6.2)

	Number of results	Mean	r_{95}	R_{95}
Beer G	8	69.8	0.599	3.619
H	8	97.2	0.638	2.339
I	9	83.3	0.451	2.734
J	9	84.1	0.366	2.751
K	9	81.0	0.436	3.028

Table 1.13 Total Nitrogen mg/l (Kjeldahl, EBC method 9.9.1)

	Number of results	Mean	r_{95}	R_{95}
Beer G	8	444	10.7	29.6
H	8	399	13.1	27.1
I	10	981	18.0	49.2
J	10	594	11.1	50.8
K	10	658	19.6	47.4

Table 1.14 Free Amino Nitrogen mg/l (EBC method 9.10)

	Number of results	Mean	r_{95}	R_{95}
Beer G	12	67	1.37	10.83
H	12	37	1.82	12.77
I	13	193	6.27	29.72
J	13	69	3.22	11.27
K	13	72	4.61	18.49

Table 1.15 Vicinal Diketones by Spectrophotometry mg/l (EBC method 9.24.1)

	Number of results	Mean	r_{95}	R_{95}
Beer G	7	0.078	0.021	0.025
H	7	0.126	0.022	0.062
I	7	0.116	0.022	0.055
J	7	0.068	0.012	0.056
K	7	0.087	0.025	0.110

This method has an acceptable reproducibility, with a mean CV_R of 5.2%.

r and R are proportional to the measured value.

3.3 Total Polyphenols (EBC method 9.11)

One laboratory did not use carboxymethylcellulose as recommended.

This method shows a mean CV_R of 10%, which is rather poor, but nevertheless meets the requirements established by *Horwitz* (3).

r and R are proportional to the measured value.

3.4 Total Carbohydrate (EBC method 9.26)

One laboratory used a water bath at $94 \pm 1^\circ\text{C}$ instead of the recommended $95 \pm 0.5^\circ\text{C}$.

This method is liable to give results with a systematic error. The mean CV_R is 8%.

r and R are proportional to the measured value.

3.5 Colour (EBC method 9.6)

As for Original Extract and Real Degree of Fermentation we have two different populations : four pale beers between 8 and 12 EBC units and one dark beer sample.

As the coefficients of variation of repeatability and reproducibility for the dark beer are not different from the pale beers', it can be assumed that precision values are proportional to the beer colour. The mean CV_R is 3.0%.

3.6 Final Attenuation (EBC method 9.7)

Method 9.7 refers to two different procedures, recommended for wort (8.6.1 and 8.6.2). Both methods give equivalent results and precision values. The mean CV_R is 1.3%, which means that both methods show a good precision.

3.7 Total Nitrogen, Kjeldahl (EBC method 9.9.1)

One laboratory gave systematically high results, which were detected as outliers at all levels by Grubb's test. The mean CV_R is 2.5%.

There is no apparent relationship between r and the measured value. The reproducibility R is around 28 mg/l for the two lowest levels (400 – 450 mg/l), and around 49 mg/l for the three highest levels (600 – 1000 mg/l). No explanation for the difference between the two groups could be found and a mean value of 41 mg/l represents the best evaluation.

3.8 Free Amino Nitrogen (EBC method 9.10)

One laboratory was detected as a straggler for reproducibility at all levels, with systematically low results. These results were eliminated.

CV_R for the 5 beers lie between 6 and 12%, which show that the method is not very precise, but nevertheless meets the requirements established by *Horwitz* (3).

r and R are proportional to the measured value.

Table 2 Beer Analysis Precision – Summary of results (m = measured value)

	Range	Number of laboratories	r_{95}	R_{95}
Alcohol % m/m (EBC 9.2.1)	1.7-7.0	11-12	0.03 + 0.005 m	0.03 + 0.02 m
Alcohol % v/v (EBC 9.2.1)	2.2-9.0	11-12	0.04 + 0.004 m	0.04 + 0.02 m
Real Extract % m/m (EBC 9.4)	3.0-6.0	10-12	0.020	0.02 m
Apparent Extract % m/m (EBC 9.4)	1.5-3.0	10-12	0.018	0.080
Original Extract % m/m (EBC 9.4)	7.0-12.0	10-12	0.07	0.19
	19.0	9	0.15	0.38
Real Degree of Fermentation % (EBC 9.5)	63-71	10-11	0.21	0.61
	50	9	0.50	1.16
Bitterness B.U. (EBC 9.8)	13-36	13	0.44 + 0.014 m	- 0.7 + 0.18 m
Total Polyphenols mg/l (EBC 9.11)	73-176	11-12	4.1	18 + 0.13 m
Total Carbohydrate g/100 ml (EBC 9.26)	2.3-4.6	8-10	0.04 + 0.02 m	- 0.2 + 0.3 m
Colour EBC units (EBC 9.6)	8-120	12-14	0.022 m	0.085 m
Final Attenuation %, refer.method (9.7 and 8.6.1)	69-98	8-9	0.45	2.62
Final Attenuation %, rapid method (9.7 and 8.6.2)	69-98	8-9	0.50	2.89
Total Nitrogen mg/l (EBC 9.9.1)	400-1100	8-10	15	41
Free Amino N mg/l (EBC 9.10)	37-200	12-13	0.7 + 0.032 m	7.2 + 0.105 m
Vicinal Diketones mg/l (EBC 9.24.1)	0.06-0.13	7	(0.02)	(0.06)

3.9 Vicinal Diketones by Spectrophotometry (EBC method 9.24.1)

Results from only 7 laboratories were obtained, because VDK's are more often measured by GC. Precision values are given only as a guide, as the number of results was slightly too low (8 laboratories are required). CV_R lie between 11 and 45% (mean 24%), which is here again in accordance with Horwitz's values for an analyte concentration of 0.1 mg/l.

There is no relationship between r or R and the measured value.

3.10 Fermentable Carbohydrate by HPLC (EBC method 9.27)

Very few (2 to 5) results were received and no statistical evaluation could be done.

4 Conclusion

The minor methodology differences reported by a few laboratories for Bitterness, Total Polyphenols and Total Carbohydrate had no influence on the results in terms of difference between duplicates or mean value.

The EBC Analysis Committee approved the results for publication in Analytica-EBC under the corresponding methods (in fact the results of the first ring test were approved in 1997 and are already appearing in Analytica-EBC).

A few beer analysis methods published in Analytica-EBC are left without any valuable precision data because these methods are not much used nowadays and it was not possible to gather enough laboratories to organize an intercollaborative test.

5 Zusammenfassung

Benard, M.: Bestimmung von Wiederholbarkeit und Reproduzierbarkeit durch EBC-erkannte Methoden: V-Bier — Monatsschrift für Brauwissenschaft 53, Nr. 5/6, 101 – 104, 2000

BC 30 Allgemeines (Braueriebetriebskontrolle)

In der Analytica-EBC veröffentlichte Methoden zur Bestimmung des Alkoholgehaltes im Bier (9.2.1), des wirklichen und scheinbaren Stammwürzegehaltes (9.4), des wirklichen Vergärungsgrades (9.5), der Farbe (9.6), des Endvergärungsgrades (9.7), der Bittere (9.8), des Gesamtstickstoffs (9.9.1), des freien Aminostickstoffs (9.10), der Gesamtpolyphenole (9.11), der vicinalen Diketone (9.24.1), der Gesamtkohlenhydrate (9.26) und der vergärbaren Kohlenhydrate (9.27) wurden in einem Ringversuch durch das Analysenkomitee der EBC, gemäß ISO Standard 5725, getestet. Werte der Wiederholbarkeit (r_{95}) und der Reproduzierbarkeit (R_{95}) werden aufgezeigt.

Benard, M.: Détermination de la répétabilité et de la reproductibilité par des méthodes reconnues par l'EBC: V-Bière — Monatsschrift für Brauwissenschaft 53, Nr. 5/6, 101 – 104, 2000

BC 30 Généralités (Contrôles de fabrication en brasserie)

Les méthodes publiées dans Analytica EBC pour la détermination de l'alcool de la bière (9.2.1), extrait réel, apparent et extrait primitif (9.4), degré réel de fermentation (9.5), couleur (9.6), atténuation limite (9.7), amertume (9.8), azote total (9.9.1), azote aminé libre (9.10), polyphénols totaux (9.11), Diccétones vicinales (9.24.1), glucides totaux (9.26), glucides fermentescibles (9.27), ont été testés par un test inter-laboratoire par le Comité des Analyses de la European Brewery Convention suivant le Standard ISO 5725 et les résultats de répétabilité (r_{95}) et de reproductibilité (R_{95}) sont présentés.

6 References

1. Benard, M., Journal of the Institute of Brewing **98**, 81, 1992.
2. ISO standard 5725-2, 1994.
3. Horwitz, W., Anal. Chem. **54**, 1, 1982.