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Report by the EBC Analysis Committee on the Determination of NDMA in Beer by GC-TEA or GC-MS Detection (2009 Collaborative Trial)

Approved by the Analysis Committee for inclusion in the EBC Analytica.

The determination of NDMA in beer by the method of GC-TEA or GC-MS was collaboratively tested by the EBC Analysis Committee according to *ISO standard 5725-2: 1994*. Repeatability (r95) and reproducibility (R95) values are presented. There is statistically significant evidence (Spearman rho = 0.83, p = 0.008) of dependence of repeatability on the mean value (repeatability is worse for bigger mean), there is also statistically significant evidence (Spearman rho = 0.94, p = 0.001) of dependence of reproducibility on the mean value (reproducibility is worse for bigger mean). This relationship between s and m can be represented by a straight line $s = a + bm$. Final repeatability standard deviation = $s_r = 0.022 + 0.034\ m$. Final reproducibility standard deviation = $s_r = 0.054 + 0.117\ m$. Note that the precision becomes poor at concentrations below 0.5 µ/l.

Descriptors: NDMA in beer, Analytics, EBC Analytica, GC-TEA-Analysis, GC-MS-Analysis

1 Introduction

The EBC Analysis Committee tested a new method for the determination of NDMA in beer that uses the principle of GC-TEA or GC-MS. An instrument – the Thermo Energy Analyser (TEA), is commercially available and is finding use within the brewing and malting industry. Beer is extracted with dichloromethane and after concentration a GC analysis is performed. A nitroso-sensitive detector (TEA or MS) is used. The NDMA concentration in the sample can be calculated with the use of calibration samples.

2 Experimental

Twelve identical bottles of each of seven beers containing prepared concentrations between 0.18 to 2.42 µg/litre of NDMA were distributed to 12 participants nominated by the EBC and ASBC Analysis Committees. Twelve sets of data were received for samples one to seven.

3 Results and discussion

Participant 5 appeared to have a significant problem with the analysis and the EBC Analysis Committee decided to eliminate their results prior to statistical evaluation. This still left sufficient laboratories presenting results to justify statistical analysis. All the data used in the statistical analysis is presented in table 1 for each sample and participant. Statistical evaluation was carried out using Mandel's k and h statistics to test the consistency of results.

- k refers to within-laboratory consistency and repeatability and the results are shown in figure 1
- h refers to between laboratory consistency and reproducibility and the results are shown in figure 2

Outliers and Stragglers

Outliers have been evaluated using the Cochran's test and stragglers using the Grubbs'test. Data have already been checked and corrected. Looking at the collation of values (standard deviations, indicating the spread of the values), we see that for the precision of sample 7 results from participants 2 and 4 are very poor. Two combinations are excluded: Participant 2, bottle 7 and participant. 4, bottle 7.

The results of these evaluations are shown in tables 2 and 3 respectively.

Estimation of repeatability and reproducibility standard deviation

- For each level, the variance (within-cell means) and mean (within-cell variances) is calculated as shown in table 4;
- For equal numbers/cell repeatability variance = s_{rj}^2 = mean (within-cell variance);

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Tables and figures see Appendix

- for 2/cell between-laboratory variance = s_{Lj}^2 = variance (within-cell means) - $s_{rj}^2/2$.

Reproducibility variance $s_{Rj}^2 = s_{rj}^2 + s_{Lj}^2$

Dependence of precision on *m*

Using the data calculated above s_j is then plotted against m_j (Fig. 3) and any dependency is sought.

The plot shows a clear functional relationship between the precision (repeatability and reproducibility) and mean.

There is statistically significant evidence (Spearman rho = 0.83, p = 0.008) of dependence of repeatability on the mean (repeatability is worse for bigger mean), there is also statistically significant evidence (Spearman rho = 0.94, p = 0.001) of dependence of reproducibility on the mean (reproducibility is worse for bigger mean). This relationship between *s* and *m* can be represented by a straight line $s = a + bm$ as shown in figure 4 and the two parameters *a* and *b* can be determined as follows:

Final repeatability standard deviation = $s_r = 0.022 + 0.034\ m$

Final reproducibility standard deviation = $s_R = 0.054 + 0.117\ m$

Statistical data

A summary of all the basic data is shown in table 5.

4 Conclusion

The determination of NDMA in beer by the method of GC-TEA or GC-MS was collaboratively tested by the EBC Analysis Committee according to ***ISO standard 5725-2:1994***.

There is statistically significant evidence (Spearman rho = 0.83, p = 0.008) of dependence of repeatability on the mean (repeatability is worse for bigger mean), there is also statistically significant evidence (Spearman rho = 0.94, p = 0.001) of dependence of reproducibility on the mean (reproducibility is worse for bigger mean).

This relationship between *s* and *m* can be represented by a straight line.

Final repeatability standard deviation: $s_r = 0.022 + 0.034\ m$ and reproducibility standard deviation: $s_R = 0.054 + 0.117\ m$. Note that the precision becomes poor at concentrations below 0.5 µ/l.

The method of GC-TEA or GC-MS for NDMA analysis is approved by the Analysis Committee for inclusion in the EBC Analytica.

Appendix

Table 1 Data for the determination of NDMA in the seven beer samples. NDMA data in µg/litre (Results)

Participant	Bottle 1	2	3	4	5	7	8
1	2.10	0.80	0.20	0.20	1.10		2.80
	1.90	0.70	0.20	0.20	1.00	N/A	2.80
2	1.40	0.50	0.20	0.20	0.80	**0.30	2.00
	1.30	0.40	0.20	0.20	0.90	0.20	2.30
3	1.60	0.70	0.20	0.30	0.90	0.50	2.30
	1.40	0.70	0.30	0.20	0.80	0.50	2.40
4	1.70	0.80	0.30	*0.40	0.90	**0.70	2.50
	1.70	0.80	0.40	0.30	1.00	0.50	2.50
6	1.57	0.60	0.13	0.15	0.91	0.39	2.62
	1.61	0.56	0.12	0.14	0.81	0.39	2.62
7	1.80	0.80	0.20	0.16	1.00	0.50	2.70
	1.60	0.70	0.18	0.18	1.20	0.50	2.50
8	2.10	0.90	0.10	0.10	1.00	0.50	2.70
	2.10	0.80	0.10	0.10	1.10	0.50	2.90
9	1.30	0.50	0.10	0.10	0.70	0.20	2.00
	1.20	0.50	0.10	0.10	0.60	0.20	1.90
10	1.40	0.60	0.20	0.20	0.80	0.40	2.30
	1.40	0.60	0.30	0.20	0.80	0.40	2.20
11	1.20	0.50	0.10	0.20	0.70	0.40	2.20
	1.20	0.50	0.20	0.20	0.80	0.40	2.20
12	1.60	0.70	0.20	0.20	0.90	0.40	2.60
	1.60	0.70	0.20	0.20	0.90	0.40	2.50

Outliers are marked ** and were excluded from the final statistical analysis.
A straggler is marked * and was included.
(NOTE: ** and * mark cells, i.e. pairs of data, not individual data)

Table 2 Cochran's test (For poorly repeatable participants within each bottle)

Different stages of Cochran test

Bottle	First test	Repeat
1	None	
2	None	
3	None	
4	None	
5	None	
7	**Partic. 4:** Outlier (p = 0.002)	**Partic. 2:** Outlier (p < 0.001)
8	None	

Identified as: (First test)
outliers (p-value < 0.01): Bottle 7, Participant 4.

In a "Repeat test":
Outliers (p-values < 0.01): Bottle 7, Participant 2.

Table 3 **Grubbs' test** (For aberrant participant within each bottle)

Different stages of Grubbs' test

Bottle	One outlying observation	Double
1	None	None
2	None	None
3	None	None
4	**Partic. 4:** Straggler (p = 0.041)	None
5	None	None
7	None	None
8	None	None

Identified as: (First test)
stragglers (p-value < 0.05): Bottle 4, Participant 4.

Table 4 **Table of mean, repeatability and reproducibility**

Bottle	p_j	m_j (overall mean)	s_{rj} (Repeatability Standard Deviation)	s_{Lj}^2	s_{Rj} (Reproducibility Standard Deviation)
1	11	1.58	0.080	0.079	0.292
2	11	0.65	0.043	0.017	0.138
3	11	0.19	0.043	0.005	0.080
4	11	0.19	0.031	0.004	0.073
5	11	0.89	0.074	0.016	0.147
7	8	0.41	0.000	0.010	0.099
8	11	2.43	0.098	0.071	0.284

Table 5 **Summary of statistical data**

Sample	**1**	**2**	**3**	**4**	**5**	**7**	**8**	**All**
Number of laboratories	11	11	11	11	11	10	11	11
Number of results	22	22	22	22	22	20	22	152
Number of laboratories retained after deletion of outliers	11	11	11	11	11	8	11	11
Number of accepted results	22	22	22	22	22	16	22	164
Mean	**1.58**	**0.65**	**0.19**	**0.19**	**0.89**	**0.41**	**2.43**	**0.91**
Repeatability standard deviation S(r)	0.075	0.044	0.028	0.028	0.052	0.036	0.104	0.053
Reproducibility standard deviation S(R)	0.239	0.131	0.077	0.077	0.159	0.102	0.339	0.160
Repeatability (r) 95% (2.83 x S(r))	0.213	0.124	0.080	0.080	0.147	0.101	0.295	**0.150**
Reproducibility (R) 95% (2.83 x S(R))	0.677	0.370	0.217	0.217	0.449	0.290	0.959	**0.453**

Repeatability and reproducibility standard deviations are proportional to measured values:

S(r) = 0.022 + 0.034 x value

S(R) = 0.054 + 0.117 x value

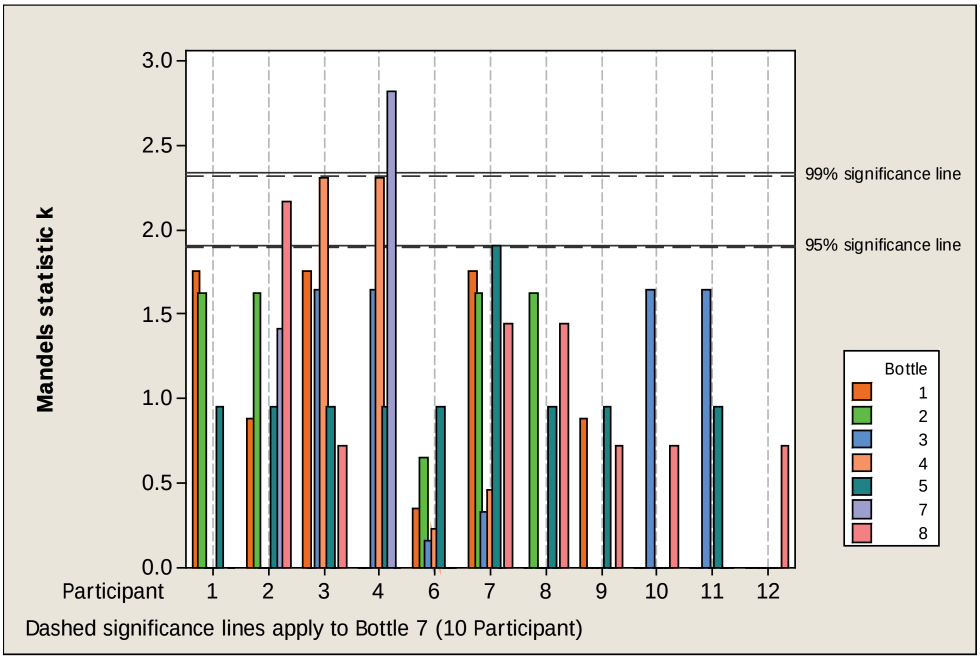

Fig. 1 Mandel's k plot grouped by Participant (within-participant consistency chart)

Mandel's k: this is looking at the variability (repeatability); whether the repeatability for one participant (for a given bottle) is bigger than average. The 95 % and 99 % significance lines are different for bottle 7 because it only has 10 participants and the other bottles have 11. Participant 4 has very poor repeatability ($p < 0.01$) for bottles 7.

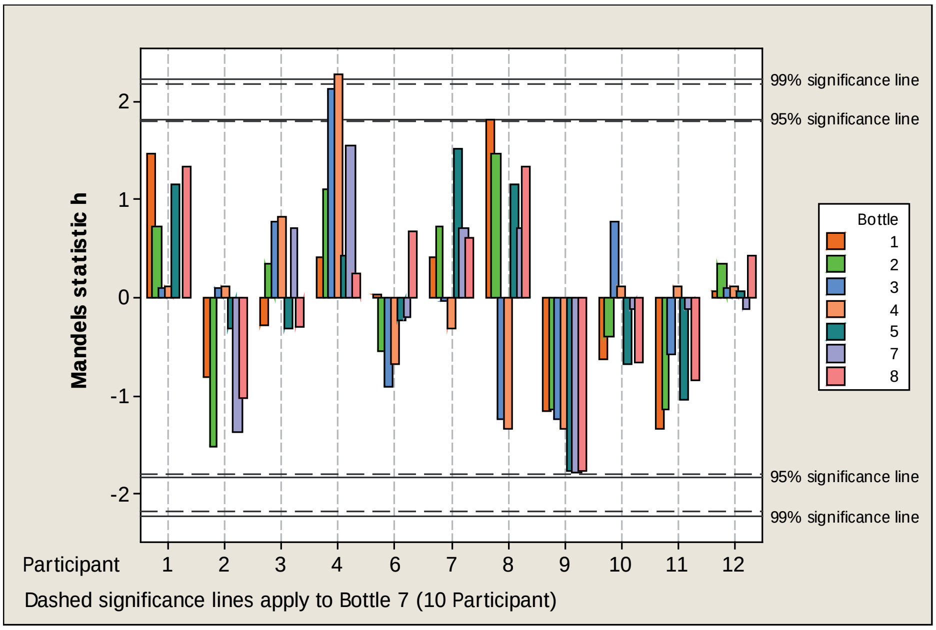

Fig. 2 Mandel's h plot grouped by participants (between-participants consistency chart)

Mandel's h: this is looking at participant bias (evaluate how similar are results obtained by different participants). The 95 % and 99 % significant lines are different for bottle 7 because it only has 10 participants and the other bottles have 11.

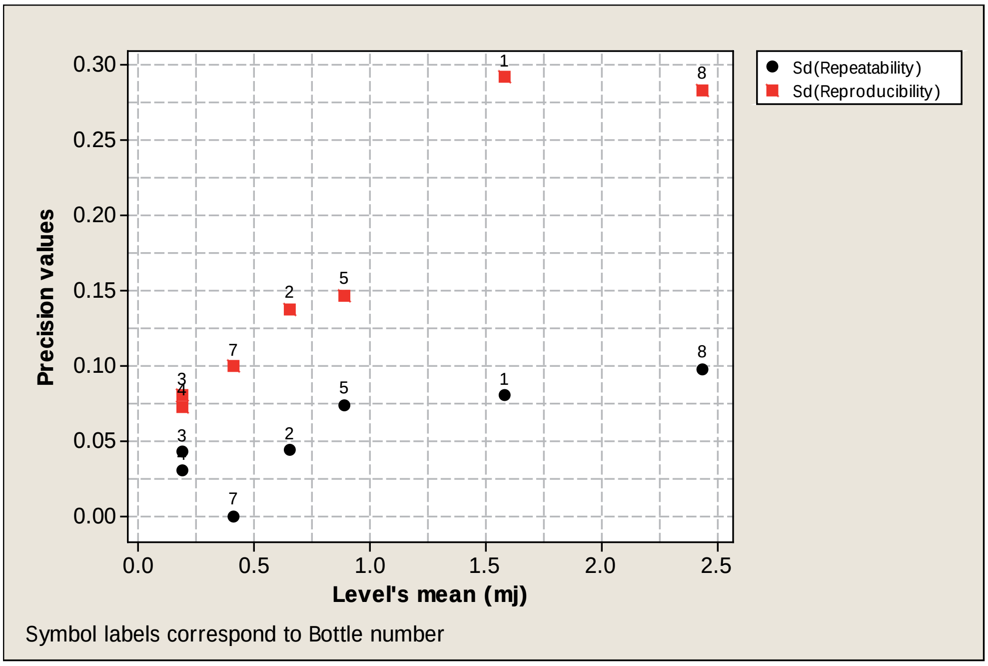

Fig. 3 Plots of s_{rj} and s_{Rj} against $\hat{m}_j$

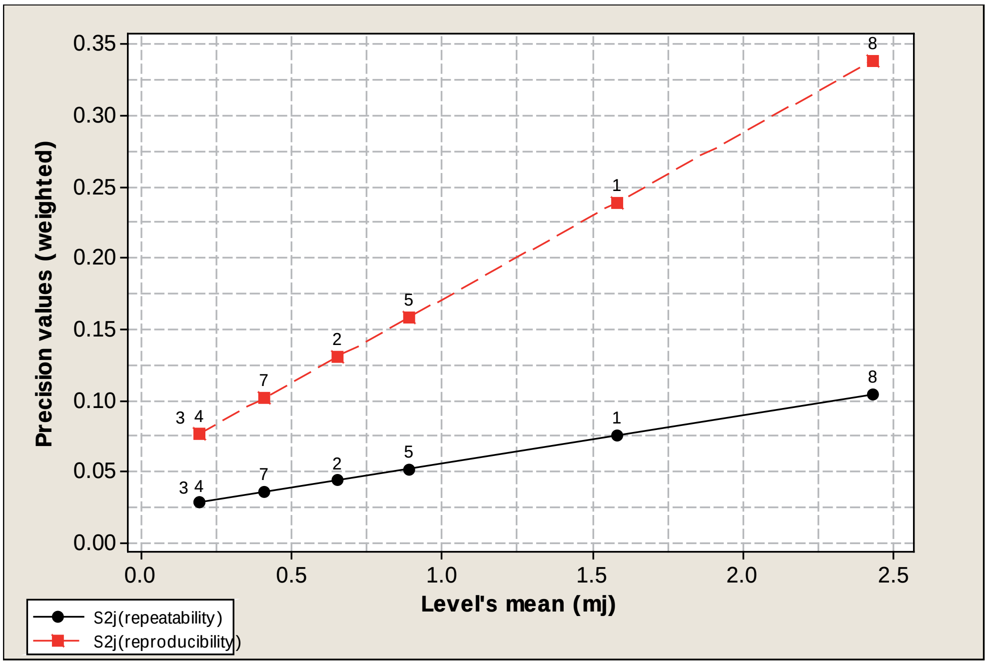

Fig. 4 Relationship between s and m