OIV-MA-AS314-04 Determination of carbon dioxide in wine by manometric method (for a range of concentration from 0.5 g/L to 7 g/L

Type II method

1. Principle

The carbon dioxide in the sample is bound with 10 M sodium hydroxide. An Erlenmeyer flask with a side arm is connected to a manometer and the carbon dioxide is released with sulphuric acid from the prepared sample. The resultant increase in pressure is measured. It allows quantifying carbon dioxide content.

2. Reagents

2.1. Freshly distilled or deionised water;

2.2. Sodium hydroxide (purity > 98%);

2.3. Sulphuric acid (purity > 95-97%);

2.4. Sodium carbonate anhydrous (purity > 99%).

Preparation of the reagents

2.5. 10 M Sodium hydroxide: dissolve 100 g of sodium hydroxide (2.2) in 200 ml water (2.1) and make up to 250 ml in a volumetric flask.

2.6. Sulphuric acid, about 50% (v/v): cautiously add concentrated sulphuric acid (2.3) to an equal volume of water (2.1). Mix well by stirring. Cool to room temperature.

2.7. Carbon dioxide standard solution 10 g/l: dry anhydrous sodium carbonate (2.4) in an oven at 260°C-270°C over night, and cool to room temperature in a desiccator. Dissolve 6.021 g of dry sodium carbonate in water (2.1) and make up to 250 ml in a volumetric flask.

2.8. Carbon dioxide calibration solutions 0.4; 1; 2; 4 and 6 g/l: with pipettes take 2, 5, 10, 20 and 30 ml of the standard solution (2.7) in separate 50 ml volumetric flasks and make up to 50 ml with water (2.1).

3. Apparatus

1. 250 ml and 50 ml volumetric flasks;
2. Oven;
3. Dessicator;

4. Balance with an accuracy of 0.1 mg;
5. Refrigerator or water-ethylene glycol bath, -4°C;
6. Electronic density meter or pycnometer and thermostatic water bath, 20°C;
7. Pipettes 0.5, 2, 3, 5, 10, 20 and 30 ml;
8. 100 ml cone-shaped vial, large ground-glass mouth;
9. Digital manometer (allowing measures up to 200 kPa with an accuracy of 0.1kPa);
10. Reaction flask: 25 ml Erlenmeyer flask with a 3 ml side arm and a three-way valve (see figure 1);
11. Vacuum system (i.e. water suction pump).
12. Separation funnel

4. Procedure

4.1. Sample preparation

Prepare the sample in duplicate. Cool the sample in a refrigerator overnight or in a -4°C water-ethylene glycol bath for 40 min. Place 3 ml of 10 M sodium hydroxide solution (2.5) in a 100 ml cone-shaped vial. Weigh the flask with contents at an accuracy of 0.1 mg. Pour approximately 75 ml of the cooled sample in the cone-shaped vial containing the sodium hydroxide solution. Weigh the flask with contents at an accuracy of 0.1 mg. Mix and allow to warm up to room temperature

4.2. Determination of carbon dioxide content

Transfer 2 ml of the prepared sample (4.1) into the reaction flask. Connect the flask to the manometer via the open three-way valve. Pipette 0.5 ml of 50% sulphuric acid (2.6) into the side arm. Secure the three-way valve and the side arm stopper with clips. Note the air pressure. Close the three-way valve. Mix the contents by tilting and shaking vigorously. Note the pressure. The prepared sample can be diluted with water if necessary.

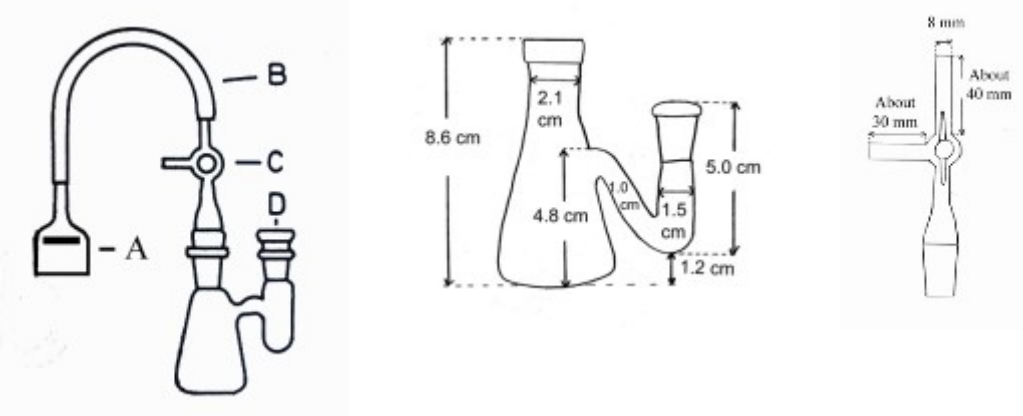


Fig.1 Apparatus. A manometer, B rubber hose, C three-way valve, D reaction flask (left) and approximate measures of the glassware (centre and right).

4.3. Calibration

Determine the carbon dioxide content of the calibration solutions as described above (4.2). Measure three calibration solutions which are within the expected concentration range of the sample. These calibration solutions are measured in duplicate.

4.4. Measurement of the density of the sample

Remove carbon dioxide from the sample by shaking the sample first in a separation funnel and then for 3 min in a vacuum generated by a water suction pump. Measure the density of the sample either with an electronic density meter or a pycnometer.

5. Calculation

Calculate the pressure increase caused by the carbon dioxide released from each calibration solution and construct a calibration graph.

Calculate the slope (a) and bias (b) of the calibration graph.

Volume V (ml) of the prepared sample:

$$V = [(m2-m1) \times 1000]/d \quad (1)$$

where

m1 (g)= weight of (flask + 3 ml NaOH);

m2 (g) = weight of (flask + 3 ml NaOH + sample);

d (kg/m^3) = density of sample.

Pressure increase p_i caused by the carbon dioxide released from the prepared sample:

$$pi = ps - pap \quad (2)$$

where

p_s = manometer reading after releasing the carbon dioxide from the sample

p_{ap} = manometer reading before addition of H_2SO_4(i.e. air pressure)

Concentration of carbon dioxide, C, in the sample (g/l) is given by:

C = [(pi - b) / a] x [(V + 3)/V] x L (3)

where

p_i = increase of pressure (equation 2)

a = slope of calibration graph

b = bias of calibration graph

V = sample volume (equation 1)

L = dilution factor in case the sample is diluted after sample preparation

Content of carbon dioxide in % by weight:

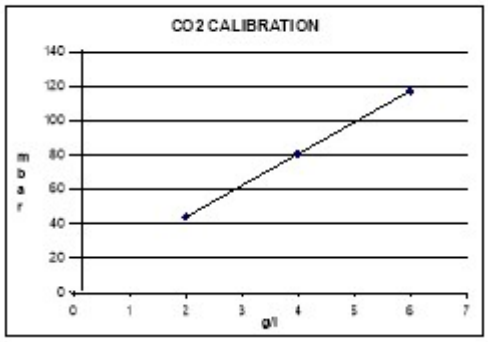
CO2 % (w/w) = C x 100/d (4)

Example of the calculation of the content of carbon dioxide:

Calibration

Conc of STD g/l	Air pressure mbar	Pressure std mbar	Pressure increase mbar
2	1021	1065	44
2	1021	1065	44
4	1021	1101	80
4	1021	1102	81
6	1021	1138	117
6	1021	1138	117

slope	18.25000
intercept	7.50000
correlation	0.99995



Calculation of the content of CO_2

SAMPLE	Density d (kg/m^3)	Flask + NaOH m1 (g)	Flask NaOH+ sampe m2 (g)	Air pressure p_{ap} (mbar)	Sample pressure p_s (mbar)	p_s-p_{ap}	Sample V (ml)	CO2 g/l	Mean CO2 g/l
Sparkling wine 1	1027.2	84.6287	156.162	1021	1112	91	69.64	4.77	
Sparkling wine 1	1027.2	84.6287	156.162	1021	1113	92	69.64	4.83	4.80
Sparkling wine 2	1025.3	86.1066	153.4407	1021	1118	97	65.67	5.13	
Sparkling wine 2	1025.3	86.1066	153.4407	1021	1118	97	65.67	5.13	5.13

6. Validation

6.1. Performance criteria

- Standard deviation estimated from duplicates, s_o = 0.07 g/l
- Relative standard deviation, RSD = 1.9%
- Repeatability, r = 5.6 %
- Expanded measurement uncertainty (k = 2), U = 3.8%
- Calibration range 0.4-6 g/L
- Determination range 0.3 -12 g/L (samples with concentration above 6 g/L should be diluted 1:2 with water to fit the calibration range)
- Detection limit 0.14 g/L

Annex A Literature

- European Brewery Convention Analytica-EBC, Fourth edition, 1987, 9.15 Carbon dioxide.
- OIV, SCMA 2002, FV N° 1153, determination of carbon dioxide in alcoholic beverages by a modified EBC method
- OIV, SCMA 2004, FV N° 1192, determination of carbon dioxide in alcoholic Beverages by a modified EBC method, Statistical results of the collaborative study
- OIV, SCMA 2005, FV N° 1222, comparison of the titrimetric method and the modified EBC method for the determination of carbon dioxide in alcoholic beverages
- Ali-Mattila, E. and Lehtonen, P., Determination of carbon dioxide in alcoholic beverages by a modified EBC method, Mitteilungen Klosterneuburg 52 (2002): 233-236

Annex B: Statistical results of the collaborative study

Determination of carbon dioxide in alcoholic beverages by a modified EBC method

1. Goal of the study

The objective of the study was to determine the repeatability and reproducibility of the modified EBC method for the determination of carbon dioxide in wines, sparkling wines, ciders and beers.

2. Needs and purpose of the study

Fermentation produces carbon dioxide in alcoholic beverages. In the production of sparkling wines, carbon dioxide is one of the most essential products and it can also be added to certain alcoholic beverages. Carbon dioxide modifies the taste and aroma and is a preserving agent in alcoholic beverages.

In accordance with the definitions of the International Code of Oenological practices, sparkling wine should have an excess pressure of not less than 3 bar due to carbon dioxide in solution, when kept at a temperature of 20°C in closed containers. Correspondingly semi-sparkling wine should have an excess pressure of not less than 1 bar and not more than 2,5 bar. Excess pressure of, 3 bar, 2.5 bar and 1 bar correspond at 20°C about, 5.83 g/L, 5.17 g/L and 3.08 g/L of carbon dioxide in solution, respectively.

There is currently no practical and reliable method for the determination of carbon dioxide in alcoholic beverages. The wide variation in carbon dioxide results in international proficiency tests is a clear indication of the fact that there is a need for a reliable method.

3. Scope and applicability

The proposed method is quantitative and it is applicable for the determination of carbon dioxide in alcoholic beverages. This method was validated in a collaborative study for the determination of carbon dioxide in wine, beer, cider and sparkling wine via the analyses at levels ranging approximately from 0.4 g/L to 12 g/L (Note: the actual calibration level ranges from 0,4 g/L to 6 g/L. The samples should be diluted with water to this level in case the carbon dioxide content is higher than 6 g/L).

4. Materials and matrices

The collaborative study consisted of 6 different samples. All except the beer samples were sent in blind duplicate, so that in total 12 bottles were distributed to the participants: two beers, two ciders, two red wines, two white wines, two pearl wines and two sparkling wines. Each bottle was coded individually for each participant. All samples were delivered in original bottles and the labels were removed from all samples except the sparkling wine samples. Measuring the amount of carbon dioxide in 10 bottles of the same lot number tested the homogeneity of the samples.

5. Practice samples

Four control samples were sent to participants to familiarize them with the method. These samples included one beer, one wine, one pearl wine and one sparkling wine

sample.

6. Method to be followed and supporting documents

The method and an Excel table for the calculation of results were sent to participants. Supporting documents were also given, including the covering letter, sample receipt form, and result sheets.

7. Data analysis

7.1. Determination of outliers was assessed by Cochran's test, Grubbs' test and bilateral Grubbs test.

7.2. Statistical analysis was performed to obtain repeatability and reproducibility data.

8. Participants

Nine laboratories in different countries participated in the collaborative study. Lab-codes were given to the laboratories. The participating laboratories have proven experience in the analysis of alcoholic beverages.

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Sweden

9. Results

The homogeneity of the samples was determined by measuring the carbon dioxide content in 10 bottles of the same lot number at the Alcohol Control Laboratory (Finland). Samples with the corresponding lot numbers were sent to the participants.

CO_2 g/L	Beer 1	Beer 2	Cider	White wine	Red Wine	Pearl wine	Sparkling wine
Means	5.191	5.140	4.817	1.337	0.595	5.254	7.463
	0.020	0.027	0.025	0.036	0.038	0.022	0.046

According to the homogeneity test the CO_2 content in the two beers was the same and

therefore they were considered as blind duplicates.

The individual results for all samples and laboratories of the collaborative study are given below.

Lab code	Beer 1	Beer 2	Cider 1	Cider 2	White wine 1	White wine 2	Red wine 1	Red wine 2	Pearl wine 1	Pearl wine 2	Sparkling wine 1	Sparkling wine 2
A	5,39	5,08	4,75	4,91	1,25	1,11	0,54	0,54	5,15	5,22	6,93	6,91
B	4,76	5,53	4,71	4,70	1,90[3]	1,78[3]	0,73[2]	1,19[2]	5,85[3]	5,93[3]	7,66[3]	7,72[3]
C	5,15	5,14	4,93	4,94	1,36	1,41	0,51	0,48	5,23	5,33	7,33	7,36
D	3,13[1]	3,95[1]	4,36[1]	0,38[1]	1,11[1]	1,11[1]	0,43[1]	0,38[1]	4,47[1]	4,29[1]	5,54[1]	5,52[1]
E	4,87	4,73	4,96	4,78	1,52	1,52	0,78[3]	0,80[3]	4,98	4,94	6,83	6,17
F	5,34	4,91	4,71	5,01	1,33	1,40	0,46	0,57	5,22	4,95	6,52	6,67
G	5,18	5,15	4,82	4,86	1,37	1,36	0,56	0,59	5,22	5,27	7,54	7,47
H	5,42	5,40	5,05	5,12	1,15	1,30	0,52	0,53	5,12	5,10	7,25	7,34
I	5,14	5,13	4,65	4,76	1,16	1,19	0,47	0,61	5,16	5,06	6,88	6,48

1. Removed because of large systematic error obviously due to poor calibration
2. Outlier by Cochran's test
3. Outlier by Grubbs' test

Statistical results of the collaborative test are summarised below.

	Beer	Cider	White wine	Red Wine	Pearl wine	Sparkling wine
Mean (g/L)	5.145	4.859	1.316	0.532	5.139	6.906
Mean rep. 1 (g/L)	5.156	4.833	1.306	0.510	5.154	6.897
Mean rep 2 (g/L)	5.134	4.885	1.327	0.553	5.124	6.914
s_r (g/L)	0.237	0.089	0.060	0.053	0.086	0.149
s_R (g/L)	0.237	0.139	0.135	0.059	0.124	0.538
SDR_r (%)	4.597	1.821	4.562	9.953	1.663	2.163
RSD_R (%)	4.611	2.855	10.22	11.07	2.407	7.795
r (2,8*s_r) (g/L)	0.662	0.248	0.168	0.148	0.239	0.418
R (2,8*s_R) (g/L)	0.664	0.388	0.377	0.165	0.346	1.507

HORRAT R	1.043	0.640	1.883	1.779	0.544	1.843

Conclusion

The Horrat values are < 2 indicating an acceptable method. The Horrat values are, however, a little bit high. In five of the nine participating laboratories these tests were made almost with no previous experience. Therefore the results can be considered at least as very satisfactory.

The method gives the results in g/L but the results can be converted to pressure units[1]

Annex C: Validation at low carbon dioxide levels

1. The detection and the determination limit

A sample of white wine was analysed in duplicate ten times. The statistical data was as follows:

Replicates	10
Mean CO_2 (g/L)	0.41
Standard deviation of the mean, s (g/L)	0.048
Detection limit 3 x s	0.14
Determination limit 6 x s	9.48

2. Standard addition

Standard additions in five different concentrations in duplicates were made into the same wine which was used for the determination of the detection and determination limits. The corresponding concentrations of CO_2 were also added to water. The linear regressions of these two experiments were compared.

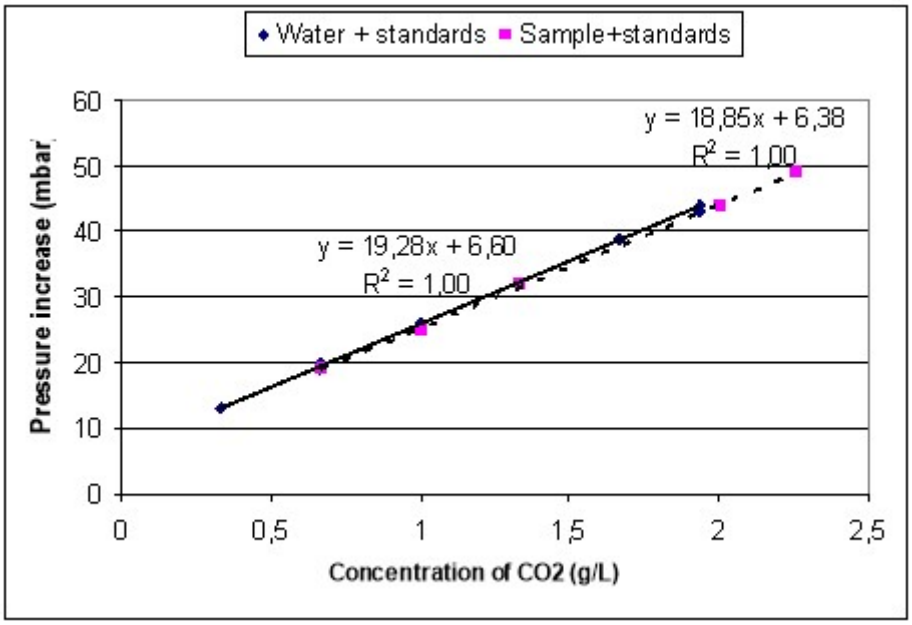

Fig. 1 Standard additions to the sample and to water

Statistical data of the plots:

	Water+ standards	Sample+ standards
Slope	19.3	18.9
Uncertainty of the slope	0.3	0.3
Intercept	6.6	6.4
Uncertainty of the intercept	0.4	0.5
Residual standard deviation	0.4	0.3
number of samples	15	10

According to statistical data the two regression lines are similar.

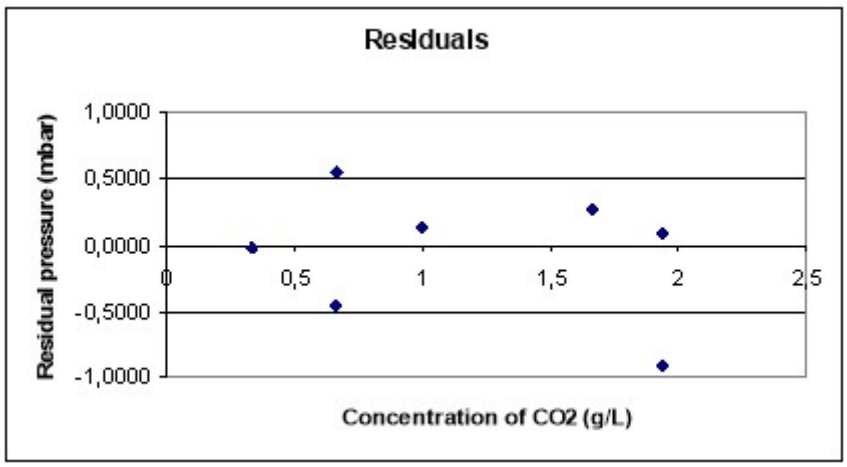

Fig. 2. The residuals of the "water+standards" equation

The residuals are dispatched on both sides of zero indicating that the regression line is linear.

Annex D: Comparison with other techniques and laboratories

1. **Comparison of the modified EBC method with the commercial Anton Paar CarboQC instrument**

Sample	Modified EBC method (g/L)	Anton Paar Carbo QC (g/L)	Difference
Sparkling wine	9,14	9,35	-0,21
Cider	4,20	4,10	0,1
White wine	1,18	1,10	0,08
Red wine	1,08	0,83	0,25
Beer 1	5,26	5,15	0,11
Beer 2	4,89	4,82	0,07
Beer 3	4,90	4,92	-0,02

Non-alcohol beer 1	5,41	5,33	0,08
Non-alcohol beer 2	5,39	5,36	0,03
			Mean 0.006

According to t-test there is no systematic difference in the measurements.

2. Comparison between Bfr, Germany and ACL, Finland

Bfr sent four samples to ACL, and ACL sent five samples to Bfr. These nine samples were analysed independently both by ACL using the method presented in this paper and in Germany at Bfr using the titrimetric method. Statistics of the results were as follows:

Mean of the difference	0.14 g/L
Std. of the difference	0.13 g/L
Z-score	1.04

The method presented here and the titrimetric method were also compared by Bundesamt für Weinbau in Austria using 21 samples of their own. Statistical data was as follows:

Mean of the difference	-0.01 g/L
Std. of the difference	0.26 g/L
Z-score	-0.03

Conclusion

According to this paper as well as earlier experiments this method is universal. It is suitable for the determination of the carbon dioxide content in all kinds of alcoholic beverages, e.g. beers, wines, fruit wines, ciders, pearl wines and sparkling wines with the concentration level of 0.3 g/L and higher.

[1] Troost, G. and Haushofer, H., Sekt, Schaum- und Perlwein, Eugen Ulmer Gmbh & Co., 1980, Klosterneuburg am Rhein, ISBN 3-8001-5804-3, Diagram 1 on the page 13.