OIV-MA-AS314-01 Carbone dioxide (with a. range of concentration up to 1.5 g/L)

Type II method

1. Principle

1.1. Still wines (CO_2 over pressure $\leq$ 0.5 x 10^5 Pa[*])

The volume of wine taken from the sample is cooled to around 0°C and mixed with a sufficient quantity of sodium hydroxide to give a pH of 10-11. Titration is carried out with an acid solution in the presence of carbonic anhydrase. The carbon dioxide content is calculated from the volume of acid needed to change the pH from 8.6 (bicarbonate form) to 4.0 (carbonic acid). A blank titration is carried out in the same conditions on decarbonated wine in order to take account of the volume of sodium hydroxide solution taken up by the wine acids.

1.2. Sparkling and semi-sparkling wines

The sample of wine to be analyzed is cooled near to the freezing point. After removal of a sub-sample to be used as a blank after decarbonation, the remainder of the bottle is made alkaline to fix all the carbon dioxide in the form of Na_2CO_3. Titration is carried out with an acid solution in the presence of carbonic anhydrase. The carbon dioxide content is calculated from the volume of acid solution needed to change the pH from 8.6 (bicarbonate form) to 4.0 (carbonic acid). A blank titration is carried out in the same conditions in decarbonated wine in order to take account of the volume of sodium hydroxide taken up by the wine acids.

2. Description of the method

2.1. Still Wines

- (CO_2 over pressure $\leq$ 0.5 x 10^5 Pa)

1. Apparatus

- Magnetic stirrer
- pH meter

2. Reagents

- Sodium hydroxide solution, 0.1 M
- Sulfuric acid solution, 0.05 M

- Carbonic anhydrase solution, 1 g/L

3. Procedure

Cool the wine sample together with the 10 mL pipette used for sampling to approximately 0°C.

Place 25 mL sodium hydroxide solution, 0.1 M, in a 100 mL beaker; add two drops of carbonic anhydrase solution, 1 g/L. Introduce 10 mL of wine using the pipette cooled to 0°C.

Place the beaker on the magnetic stirrer, immerse the pH electrode and magnetic rod, and stir moderately.

When the liquid has reached room temperature, titrate slowly with the sulfuric acid solution, 0.05 M, until the pH reaches 8.6. Note the burette reading.

Continue titrating with the sulfuric acid until the pH reaches 4.0. Let *n* mL be the volume used between pH 8.6 and 4.0.

Remove CO_2 from approximately 50 mL of the wine sample by shaking under vacuum for three minutes, the flask being heated in a water bath to about 25 °C.

Carry out the above procedure on 10 mL of the decarbonated wine. Let *n*'mL be the volume used.

2.1.4. Expression of results

1 mL of the titrated sodium hydroxide solution, 0.05 M, corresponds to 4.4 mg of CO_2.

The quantity of CO_2 in grams per liter of wine is given by:

- 0.44 (*n* - *n*')

The result is quoted to two decimal places.

Note: For wines which contain little CO_2 (CO_2 < 1 g/L), the addition of carbonic anhydrase to catalyze the hydration of CO_2 is unnecessary.

2.2. Sparkling and semi-sparkling wines

2.2.1. Apparatus

- Magnetic stirrer
- pH meter

2. Reagents

- Sodium hydroxide, 50% (*m/m*)
- Sulfuric acid solution, 0.05 M

- Carbonic anhydrase solution, 1 g/L

3. Procedure

Mark the level of wine in the bottle and then cool until freezing begins.

Allow the bottle to warm up slightly, while shaking, until ice crystals disappear.

Remove the stopper rapidly and place 45 to 50 mL of wine in a measuring cylinder for blank titration. The exact volume removed, *v* mL, is determined by reading on the measuring cylinder after it has returned to room temperature.

Immediately after the blank sample has been removed, add 20 mL of the sodium hydroxide solution for a 750 mL bottle.

Allow the wine to reach room temperature.

Place 30 mL of boiled distilled water and two drops of the carbonic anhydrase solution into a 100 mL beaker. Add 10 mL of wine that has been made alkaline.

Place the beaker on the magnetic stirrer, set up the electrode and magnetic rod and stir moderately.

Titrate with the sulfuric acid solution, 0.05 M, slowly until the pH reaches 8.6. Note the burette reading.

Continue titrating slowly with the sulfuric acid, 0.05 M, until the pH reaches 4.0. Let *n* mL be the volume added between pH 8.6 and 4.0.

Remove CO_2 from the *v* mL of wine placed on one side for the blank titration by agitating under vacuum for three minutes, the flask being heated in a water bath at about 25 °C. Remove 10 mL of decarbonated wine and add to 30 mL of boiled distilled water, add two to three drops of sodium hydroxide solution, 50%, to bring the pH to 10 to 11. Then follow the above procedure. Let *n*' mL be the volume of sulfuric acid added, 0.05 M.

2.2.4. Expression of results

1 mL sulfuric acid, 0.05 M, corresponds to 4.4 mg of CO_2.

Empty the bottle of wine which has been made alkaline and determine to within 1 mL the initial volume of wine by making up to the mark with water, say V mL. The quantity of CO_2 in grams per liter of wine is given by the following formula:

$$0.44\,(n - \frac{V - v + 20}{V - v}$$

The result is quoted to two decimal places.

2.3. *Expression of Results*

The excess pressure at 20°C ($Paph_{20}$) expressed in Pascals is given by the formula:

$$Paph_{20} = \frac{Q}{1.951 \times 10^{-5}(0{,}86 - 0{,}01A)(1 - 0{,}00144S)} - Patm$$

Where :

- Q = CO_2 content in g/L of wine,
- A= the alcoholic strength of wine at 20 °C,
- S= the sugar content of the wine in g/L,
- Patm = the atmospheric pressure, expressed in Pascals.

2.4. *Note*

The procedure described below can be used as the usual method for wines containing less than 4 g per liter of carbon dioxide.

Prepare two samples of wine for analysis.

Open one of the samples after it has been cooled to approximately 5°C and immediately add 5 mL of a sodium hydroxide solution, 50% (*m/m*), for 375 mL of sample. Stopper immediately and mix. Place 10 mL of wine so processed into a beaker containing 40 mL of water and add 3 drops of carbonic anhydrase solution, 0.1 mg/mL. Titrate with a sulfuric acid solution, 0.02275 M, until reaching a pH of 8.6, then continue titrating to a pH of 4.0. The volume used to change the pH from 8.6 to 4.0 is *n* mL.

Remove the carbon dioxide from about 25 mL of wine, taken from the second sample, by agitation under a vacuum for about 1 min. into a 500 mL flask containing 3 drops of carbonic anhydrase solution. Add 0.33 mL of sodium hydroxide, 50% (*m/m*). Apply the above titration procedure to 10 mL decarbonated wine. Let *n*' mL be the volume of H_2SO_4, 0.02275 M used. 1mL corresponds to 200 mg of carbon dioxide per liter. The amount of wine analyzed for carbon dioxide, in milligrams per liter:

- (*n* - *n*') x 200 x 1.013

Bibliography

Reference method:

- Caputi A, Ueda M., Walter P. & Brown T., *Amer. J. Enol. Vitic.,* 1970, 21, 140-144.
- Sudraud P., *F.V., O.I.V.,* 1973, n° 350.

- Goranov N., *F.V., O.I.V.,* 1983, n° 758.
- Brun S. & Tep Y., *F.V., O.I.V.,* 1981, n° 736 & 1982, n° 736 (bis).

Collaborative Study Titrimetric determination of carbon dioxide in sparkling and semi-sparkling wines

Report on Results

Goal of the study

The objective of the study is to determine the repeatability and reproducibility characteristics of the reference method (MA-E-AS314-01-DIOCAR) for the titrimetric CO_2 determination in sparkling and semi-sparkling wine.

O.I.V. definitions and limits for the CO_2 content are given with resolution OENO 1/2002.

Needs and purpose of the study

The reference method for the CO_2 determination includes no precision data. This collaborative trial was thus conducted.

Due to the analytical particularity, the conventional validation protocol was not able to be completely respected. Out of one bottle of sample only one independent determination could be done. Each bottle had to be considered as individual. Therefore homogeneity testing within the pre-investigations for collaborative studies was impossible. In order to provide homogenous test material close co-operation with producers was necessary. Samples were obtained during the filling of the bottles on the filling line in a very short time space, thus that it must be assumed that the CO_2 is homogeneously distributed in all bottles.

This study was designed to be a blind duplicate test. The complete anonymity of the samples could not be guaranteed because the partners involved used different types of bottles and/or stoppers for the different samples. Therefore we had to rely on the honesty of the participating laboratories which were requested to perform the data analysis independently without any data modification.

Scope and applicability

1. The method is quantitative.
2. The method is applicable for the determination of CO_2 in sparkling and semi-sparkling wines to check that standards are respected.

Materials and matrices

The collaborative study included 6 different samples. All were sent in blind duplicate,

so that in total 12 bottles were distributed to the participants.

Table 1. Samples and coding.

Sample	Bottle Code	Type
SAMPLE A	(Code 1 + 9)	sparkling wine
SAMPLE B	(Code 2 + 5)	semi-sparkling wine ("petillant")
SAMPLE C	(Code 3 + 4)	sparkling wine
SAMPLE D	(Code 6 + 10)	semi- sparkling wine ("petillant")
SAMPLE E	(Code 7 + 11)	semi- sparkling wine ("petillant")
SAMPLE F	(Code 8 + 12)	sparkling wine (red)

Control measures

The method considered is already approved in practice. Only the missing precision data had to be determined within the collaborative study. A pre-trial was not required because most of the laboratories had been already using the reference method in routine analysis.

Method to be followed and supporting documents

Supporting documents were given to the participants (Covering letter Reference for method of analysis, Sample Receipt Form and Result Sheet).

The determination of CO_2 content in g/l should be expressed in g/l.

Data analysis

1. Determination of outliers was assessed by Cochran, Grubbs and paired Grubbs tests.
2. Statistical analysis was performed to obtain repeatability and reproducibility data.
3. HORRAT values were calculated.

Participants

13 laboratories from several different countries participated in the collaborative study. Lab-Code numbers were given to the laboratories. The participating laboratories have proven experience in the analysis of CO_2 in sparkling wine.

Table 2. List of participants.

Landesuntersuchungsamt D-56068 Koblenz GERMANY	Institut für Lebensmittelchemie und Arzneimittelprüfung D-55129 Mainz GERMANY
Landesuntersuchungsamt D-67346 Speyer GERMANY	Institut für Lebensmittel, Arzneimittel und Tierseuchen D-10557 BERLIN GERMANY
Servicio Central de Viticultura y Enologia E-08720 Villafranca Del Pendes SPAIN	Landesuntersuchungsamt D-54295 Trier GERMANY
Landesuntersuchungsamt D-85764 Oberschleißheim GERMANY	Instituto Agrario di S. Michele I-38010 S. Michele all Adige ITALIA
Chemisches Landes- u. Staatl. Veterinäruntersuchungsamt D-48151 Münster GERMANY	Ispettorato Centrale Repressione Frodi I-31015 Conegliano (Treviso) ITALY
Bundesamt für Weinbau A-7000 Eisenstadt AUTRIA	BgVV D-14195 Berlin GERMANY
Chemisches und Veterinäruntersuchungsamt D-70736 Fellbach GERMANY	

[*] 1 bar = 10^5 Pascal (Pa)