OIV-MA-AS4-02D Detection of preservatives and fermentation inhibitors

Type IV method

1. Examination and determination of ethyl pyrocarbonate (diethyl dicarbonate)

1.1. Principle

The diethyl carbonate formed by degradation of ethyl pyrocarbonate (diethyl ester of pyrocarbonic acid) in the presence of ethanol is extracted from wine using carbon disulfide and the quantity determined by gas chromatography. Either of the procedures described below may be used.

1.2. *Apparatus*

1.2.1. Gas chromatography with flame ionization detector.

1.2.2. Columns:

- Capillary column coated with Carbowax 1540
- Column length: 15.24 m
- Inside diameter: 0.51 mm
- Polypropyleneglycol on Celite 545 (15:100), 60-100 mesh
- Column length: 2 m
- Interior diameter: 3 mm

3. Reagents

1. Anhydrous sodium sulfate
2. Carbon disulfide

The carbon disulfide must contain no impurities in the critical retention zone (5 to 7 min.) for maximum sensitivity in accordance with the conditions of gas chromatography as indicated in paragraph 1.4.2.

1.4. Procedure

1.4.1. Use of the capillary column.

Place 100 mL wine in a 250 mL separating funnel with 1 mL of carbon disulfide (1.3.2). Mix vigorously for 1 min. The carbon disulfide phase separated is rapidly centrifuged,

then dried with anhydrous sodium sulfate (1.3.1).

Inject 10 µl of the clear liquid supernatant into the chromatograph.

Chromatography conditions:

Detector gases:

- hydrogen: 37 mL/min.
- air: 250 mL/min.

Gas flow:

- nitrogen: 40 mL/min.
- A 1/10 splitter sends to the detector the gas mixture with a flow rate of 3 to 5 mL/min.
- Temperature:
- injector: 150 °C; oven: 80 °C; detector: 150 °C

Detection limits:

- 0.05 mg/L of wine

1.4.2. Use of the column for polypropyleneglycol.

Add 20 mL of wine and 1 mL of carbon disulfide (1.3.2) into a conical centrifuge tube with a stopper. Agitate vigorously for 5 minutes, then centrifuge for 5 minutes applying a centrifugal force of 1000 to 1200 g. The liquid supernatant produced is aspirated by a thin-tipped pipette; the carbon disulfide phase is dried with a small quantity of anhydrous sodium sulfate, added while stirring with a glass rod. Inject 1 µL of the clear liquid into the gas chromatograph.

Chromatography conditions.

Detector gas:

- hydrogen: 35 mL/min.
- air: 275 mL/min.

Carrier gas flow:

- nitrogen: 25 mL/min.

Temperature:

- injector: 240 °C

- oven: 100 °C
- detector: 240 °C

Sensitivity range:

- 12 x 10-11 A to 3 x 10-11 A

Chart speed:

- 1 cm/min.

Detection limit:

- 0.10 - 0.05 mg/L of wine

Under these exact conditions, diethyl carbonate displays a retention time of about 6 min.

The calibration of the apparatus is carried out using solutions of 0.01 and 0.05% (*m/v*) diethyl carbonate in carbon disulfide (1.3.2).

1.5. Calculation

Quantitative determination of diethyl carbonate is carried out preferably using the internal standard method, referring to the peaks of the *iso*-butyl alcohol or *iso*-amyl alcohol which are close to that of diethyl carbonate.

Prepare two samples of test wine: one of wine with 10 mL 10% ethanol (*v/v*) added, the other the same wine to which has been added 1 mg diethyl carbonate per liter using 10 mL of a 100 mg/L solution of diethyl carbonate in 10% ethanol (*v/v*).

Treat these two samples according to one or the other of the techniques above according to the column used.

Let:

S = the peak area of the diethyl carbonate in the spiked wine

S_x = the peak area of the diethyl carbonate in the wine,

i = the peak area of internal standard in the wine,

I = the peak area of internal standard in the spiked wine .

The concentration of diethyl carbonate in mg/L of wine is:

$$\frac{S_x}{S \times \frac{i}{I} - S_x}$$

In the case where standardization is carried out using a pure standard solution of diethyl carbonate, it is necessary to predetermine the yield of the extraction with

carbon disulfide in accordance with the procedure utilized. This yield is expressed by the extraction factor F, with a decimal number less than or equal to 1 (yield 100%).

Let:

S_x= the peak area of diethyl carbonate given by the wine,

S_e= the peak area given by the injection of the same volume of a standard solution of diethyl carbonate of concentration C in mg/L,

V_x= the volume of wine used in the extraction with carbon disulfide,

V_s= the volume of carbon disulfide used for the extraction,

E_e= the sensitivity for the recording of S_x

The concentration of diethyl carbonate in mg/L of wine is:

$$\frac{C \times S_x \times E_x \times V_s}{S_e \times E_e \times F \times V_x}$$

If the concentration of the two solutions injected in the chromatograph is similar, the response is the same for the recording of Sx and of Se; the formula is simplified and becomes:

$$\frac{C \times S_x \times V_s}{S_e \times F \times V_x}$$

Bibliography

- Kielhofer E., Wurdig G., *Dtsch. Lebensmit. Rdsch.,* 1963, 59, 197-200 & 224-228.
- Prillinger F., *Weinberg u. Keller,* 1967, 14, 5-15.
- Reinhard C., *Dtsch. Lebensmit. Rdsch.,* 1967, 5, 151-153.
- Bandion F., *Mitt. Klosterneuburg, Rebe u. Wein,* 1969, 19, 37-39.