Method OIV-MA-BS-24 : R2009

Type IV method

Determination of ^{14}C content by liquid scintillation spectrometry in spirit drinks of viti-vinicultural origin

OENO 6/94;

OIV/OENO 382A/2009

Introduction

Since alcohol can be used for the development of certain alcoholic beverages it is necessary to verify its agricultural or fossil origin.

1. Procedure for determining the type of alcohol

The determination of ^{14}C content in ethanol can be used to distinguish between alcohol made with fossil raw materials (referred to as synthetic alcohol) and alcohol made with existing raw materials (referred to as fermentation alcohol).

2. Definition

The expression ^{14}C content in ethanol means the ^{14}C content determined by the specified method. The natural content in the atmosphere of ^{14}C (reference value) which is absorbed by living plants is not a constant value. Accordingly, the reference value is determined on the basis of ethanol taken each time from raw materials grown in the most recent vegetation period. This annual reference value is determined each year by collaborative analyses organised by the Community Bureau of References and the Joint Research Centre, Ispra.

3. Principle

^{14}C content is determined directly by liquid scintillation counting in samples containing alcohol with at least 85% (by mass) of ethanol.

4. Reagents

4.1. Toluene scintillator.

5.0 g 2,5-diphenyloxazole (PPO).

0.5 g p-p-bis [4-methyl-5-phenyloxazole (2)]-benzene (dimethyl-POPOP) in one litre of pure toluene.

Toluene scintillators of this composition, commercially available, ready to use, can also be used.

4.2. Standard ^{14}C.

^{14}C n-hexadecane with an activity of about 1 x 106 dpm/g (approximately 1.67. 106 cBq/g) and a guaranteed accuracy of the determined activity of $\pm$ 2% rel.

4.3. Ethanol free of ^{14}C. Synthetic alcohol made from raw materials of fossil origin with at least 85 wt% ethanol to determine the zero effect.

4.4. The reference solution i.e. alcohol made with current raw materials from the most recent vegetation period with at least 85 wt% ethanol.

5. Apparatus

5.1. Multichannel liquid scintillation spectrometer with a computer and automatic external standardization and indication of the conditions of the external standard channel (usual design: three meter channels and two external standard channels).

5.2. Low-potassium counter tubes suitable for the spectrometer, with dark screw-tops containing a polyethylene insert.

5.3. Volumetric pipettes, 10 ml.

5.4. Automatic dosing device, 10 ml.

5.5. 250 ml round-bottom flask.

5.6. Alcohol distillation apparatus with heating mantle, e.g. Micko type.

5.7. Microlitre syringe 50 µl.

5.8. Pycnometers, 25 ml and 50 ml. Pycnometer funnel

5.9. Thermostat at a constant temperature ± 0.01°C.

5.10. Alcoholometric tables to international standards.

6. Procedure

6.1. Adjusting the apparatus.

The apparatus should be adjusted according to the manufacturer's instructions.
Measuring conditions are optimal when the value E2/B, the quality index, is at its maximum.

- E = efficiency

- B = background

Only two meter channels are optimised. The third is left fully open for control purposes.

6.2. Selection of counter tubes.

A larger number of counter tubes than will be needed later are each filled with 10 ml of ^{14}C-free synthesis ethanol and 10 ml of toluene scintillator. Each is measured for at least × 100 minutes. Tubes whose backgrounds vary by more than ± 1 % rel. from the mean are discarded. For the selection, only new tubes from the factory and from the same batch may be used.

6.3. Determination of the external standard/channel ratio (ESCR).

When setting the channel provided for in point 6.10, at the same time, when calculating the efficiency coefficient using the corresponding computer program, the external standard channel ratio (CEGN) is also determined. The external standard used is 137 caesium, which is already built-in by the manufacturer..

6.4. Preparation of sample

Samples having an ethanol content of at least 85 % mass and free from impurities, which absorb at wavelengths below 450 nm can be measured. The low residue of esters and aldehydes has no disruptive effect. After the first few ml have been discarded, the sample is distilled direct into the pycnometer and the alcohol content of the sample is determined by pycnometry. The values to be determined are taken from the Official Alcohol Tables.

7. Measurement of samples using an external standard

7.1. The low absorbance samples as described in section 6.4 with an ESCR value of approximately 1.8 can be measured

through the external standard channel ratio, which provides a measure of the efficiency ratio.

7.2. Procedure

Using a pipette, introduce into a counter tube checked (selected) for background noise 10 ml of the sample prepared in accordance with section 6.40 and then each time add 10 ml of toluene scintillator to using an automatic dosing device. The samples are homogenized in the calibration bottle by rotation, taking care to ensure that the liquid does not wet the polyethylene insert in the screw-top. A tube containing ^{14}C-free fossil ethanol is prepared in the same way to measure the background noise. To check the relevant annual ^{14}C value a duplicate of recent ethanol from the latest vegetation period is prepared, mixing a counter tube with an internal standard, in accordance with paragraph 8.

The control and background samples are placed at the beginning of the measurement series, which should contain no more than 10 samples for analysis. Total measuring time per sample is at least 2 × 100 minutes, with the individual samples being measured in partial stages of 100 minutes in order to detect any equipment drift or other defect. (One cycle therefore corresponds to a measuring interval of 100 minutes per sample). Background and control samples should be freshly prepared every four weeks. This method requires little time and material and is particularly suitable for experienced laboratories processing large numbers of samples.

For samples with low absorbance (external standard channel ratio of about 1.8) the efficiency is only slightly affected by the change in this value. When this change does not exceed ± 5% rel., the calculation can be done with the same efficiency. For samples with higher absorbance such as for denatured alcohols, the efficiency can be established via the extinction correction graph. If an appropriate computer program is not available the internal standard must be used, and this gives an unambiguous result.

8. Measuring samples using internal standard hecadecane ^{14}C

8.1. Procedure

Control and background samples (recent and fossil ethanol) and the unknown material are each measured as duplicates. One sample of the duplicate is prepared in a non-selected tube and an accurately dosed quantity (30 µl) of hexadecane 14C is added as an internal standard (added activity around 26,269 dpm/gC, approximately 43,782 cBq/gC). For the sample preparation and measuring time of the other samples see 7.20, but the measuring time for the samples with the internal standard can be reduced to about five minutes by presetting at 105 pulses. One duplicate each of background and control samples is used per measuring series; these are placed at the beginning of the measuring series.

8.2. Use of internal standard and counter tubes

To prevent contamination when measuring with the internal standard these must be stored and handled well away from the area where the samples for analysis are prepared and measured. After measurement the tubes checked for background may be re-used. The screw-tops and tubes containing the internal standard are disposed of.

9. Expression of results

9.1. The unit of activity of a radio-active substance is the Becquerel. 1 Bq = 1 decay/sec.

The indication of specific radio-activity is expressed as Becquerels relative to one gram carbon = Bq/gC.

To obtain more practical results it is best to express the results in centi-bequerels: cBq/gC.

The descriptions and formulae used in the literature, based on dpm, may be retained for the time being. To obtain corresponding figures in cBq merely multiply the dpm figure by 100/60.

9.2. Expression of results with an external standard

- (cpmpr - cpmNg) . 1,918. 100 cBq/g C = V. F. Z. 60

9.3. Expression of results with an internal standard

- (cpmpr - cpmNg) . dpmis . 1,918. 100 cBq/g C = (cpmis - cpmpr) . V. F. 60

9.4. Abbreviations

- cpmpr = the mean sample count rate over the total measuring time.
- cpmNg = the mean background pulse rate calculated in the same way.
- cpmis = count rate of samples, with an internal standard.
- dpmls = the quantity of internal standard added (calibration radioactivity dpm).
- V = the volume of the samples used in ml.
- F = the content in grams of pure alcohol per ml corresponding to its concentration.
- Z = the efficiency corresponding to the ESCR value.
- 1.918 = the number of grams of alcohol per gram of carbon.

10. Repeatability of the method

10.1. Repeatability (r)

- r = 0.632 cBq/g C ; S(r) = ± 0.223 cBq/gC

10.2. Reproducibility (R)

- R = 0.821 cBq/g C ; S(R) = ± 0.290 cBq/gC.

11. Bibliography

1. Methods of analysis of neutral alcohol applicable in the wine sector. EEC Regulation No. 1238/92 (8 May 1992), EEC Regulation No. 2009/92 (20 July 1992).