

EN

ANNEX

The Annex to Regulation (EC) No 2870/2000 is amended as follows:

- (1) in the table of contents, the following point is added:
‘XI. Determination of ^{14}C content in ethanol’;
- (2) in point 5 (Reagents and Materials) of part III.2 (Gas chromatographic determination of volatile congeners: aldehydes, higher alcohols, ethyl acetate and methanol) of Chapter III, the following points are inserted:
‘5.13a For ethyl alcohol of agricultural origin only, ethanol absolute (CAS 64-17-5).’;
‘5.14.1a For ethyl alcohol of agricultural origin only, standard solution A shall be prepared pipetting the reagents with reduced volumes of higher alcohols with the purpose of having standard solutions with concentrations close to the legal limits for ethyl alcohol of agricultural origin.’;
‘5.14.2a For ethyl alcohol of agricultural origin only, standard solution B shall be prepared pipetting a suitable internal standard with the reduced volumes with the purpose of having standard solutions with concentrations close to the legal limits for ethyl alcohol of agricultural origin.’;
- (3) the following Chapter XI is added:

‘XI. DETERMINATION OF ^{14}C CONTENT IN ETHANOL

1. Introduction

Determination of the ^{14}C content in ethanol permits a distinction to be made between alcohol from fossil fuels (synthesis alcohol) and alcohol from recent raw materials (fermentation alcohol).

2. Definition

The ^{14}C content of ethanol shall be considered as the ^{14}C content determined using the method described here or the method described in standard EN 16640 Method C.

The natural ^{14}C content in the atmosphere (the reference value), which is absorbed by living vegetation by assimilation, is not a constant value. The

reference value is therefore determined on ethanol from raw materials of the most recent vegetation period. This annual reference value is determined according to standard EN 16640. However, another reference value can be accepted where it is certified by an accredited body.

3. **Principle**

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

4. **Reagents**

4.1. Toluene scintillator

5,0 g 2,5-diphenyloxazole (PPO)

0,5 g p-bis-[4-methyl-5-phenyloxazolyl(2)]-benzene (dimethyl-POPOP) in 1 litre analytical grade toluene.

Commercial, ready-to-use toluene scintillators of this composition may also be used.

4.2. ^{14}C standard

n-Hexadecane ^{14}C with an activity of about 1×10^6 dpm/g (approximately $1,67 \times 10^6$ cBq/g) and a guaranteed accuracy of determined activity of ± 2 % rel.

4.3. ^{14}C -free ethanol

Synthesis alcohol from raw materials of fossil origin with at least 85 % mass ethanol, to determine the background.

4.4. Alcohol from recent raw materials of the most recent vegetation period with at least 85 % mass ethanol as reference material.

5. **Apparatus**

- 5.1. Multi-channel liquid scintillation spectrometer with processor and automatic external standardisation and display of the external standard/channel ratio (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 with ground-glass stopper.
- 5.6. Alcohol distillation apparatus with heating mantle, e.g. type Micko.
- 5.7. Microliter syringe 50 μ l.
- 5.8. Pycnometer funnel, pycnometers, 25 ml and 50 ml. In alternative, equivalent equipment, such as electronic densimetry, should be allowed.
- 5.9. Thermostat with a temperature stability of $\pm 0,01$ $^{\circ}$ C.

6. **Procedure**

6.1. Adjusting the equipment

The equipment shall be adjusted according to the manufacturer's instructions. Measuring conditions are optimal when the value E_2/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 later be needed are each filled with 10 ml of ^{14}C -free synthesis ethanol and 10 ml toluene scintillator. Each is measured for at least $\times 100$ minutes. Tubes whose backgrounds vary by more than ± 1 % rel. from the mean are discarded. Only tubes new from the factory and from the same batch may be used.

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

During the process of setting the channels (point 6.1) the ESCR is determined using the appropriate computer program when the efficiency is determined. The external standard used is $^{137}\text{caesium}$, 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 may be measured. The low residue of esters and aldehydes has no disruptive effect. The alcohol content of the sample is previously determined with an approximation of 0,1 %.

7. **Measurement of samples using external standard**

7.1. Slightly extinguished samples such as those described in point 6.4 with an ESCR of around 1,8 may be measured via the ESCR, which provides a measure of the efficiency.

7.2. Measurement

10 ml each of the samples prepared according to point 6.4 is pipettes into a selected counter tube checked for background and 10 ml of toluene scintillator is added via an automatic dosing device. The samples in the tubes are homogenised by suitable rotary movements; the liquid shall not be allowed to 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. Check the relevant annual ^{14}C value a duplicate of recent ethanol from the latest

vegetation period is prepared, a tube being mixed with internal standard, see point 8.

The control and background samples are placed at the beginning of the measurement series, which shall 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 part stages of 100 minutes so that any equipment drift or other defect can be detected. (One cycle therefore corresponds to a measuring interval of 100 minutes per sample).

Background and control samples shall be freshly prepared every four weeks.

In the case of slightly extinguished samples (ESCR circa 1,8) the efficiency is only negligibly affected by the change in this value. If the change is within ± 5 % rel. the same efficiency can be expected. For more greatly extinguished samples, such as denatured alcohols, the efficiency may be established via the extinction correction graph. If an appropriate computer program is not available the internal standard shall be used, and this gives an unambiguous result.

8. **Measuring samples using internal standard hexadecane¹⁴C**

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 μ l) of hexadecane¹⁴C is added as 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 point 7.2, but the measuring time for the samples with the internal standard may be reduced to about five minutes by presetting at 10^5 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. Handling the internal standard and counter tubes

To prevent contamination when measuring with the internal standard these shall 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 shall be disposed of.

9. Expression of the results

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

Indication of specific radio-activity is expressed as becquerels relative to one gram carbon = Bq/gC.

To obtain more practical results, these shall be expressed in centi-bequerels = cBq/gC.

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

- 9.2. Expression of results with external standard

$$\text{cBq/g C} = \frac{(\text{cpm}_{\text{pr}} - \text{cpm}_{\text{NE}}) \cdot 1,918 \cdot 100}{V \cdot F \cdot Z \cdot 60}$$

- 9.3. Expression of results with internal standard

$$\text{cBq/g C} = \frac{(\text{cpm}_{\text{pr}} - \text{cpm}_{\text{NE}}) \cdot \text{dpm}_{\text{IS}} \cdot 1,918 \cdot 100}{(\text{cpm}_{\text{IS}} - \text{cpm}_{\text{pr}}) \cdot V \cdot F \cdot 60}$$

- 9.4. Abbreviations

cpm_{pr} = the mean sample count rate over the total measuring time.

cpm_{NE} = the mean background pulse rate calculated in the same way.

cpm_{IS} = the amount of added internal standard added (calibration radioactivity dpm).

dpm_{is} = the quantity of internal standard added (calibration radioactivity dpm).

V = the volume of the samples used in ml.

F = the content in grammes pure alcohol per ml corresponding to its concentration.

Z = the efficiency corresponding to the ESCR value.

1,918 = the number of grammes alcohol per gramme carbon.

10. **Reliability of the method**

10.1. Repeatability (r)

$$r = 0,632 \text{ cBq/g C}; S_{(r)} = \pm 0,223 \text{ cBq/g C}$$

10.2. Reproducibility (R)

$$R = 0,821 \text{ cBq/g C}; S_{(R)} = \pm 0,290 \text{ cBq/g C.}$$