Customs Laboratories
European Network

ILIADe 143:2023 | CLEN Method

Determination of Ethanol in Alcoholic Products by GC-FID

Version 25 July 2023

This table shows the most important changes that have been made compared with the latest former version	
Date of the latest former version: 2 February 2021	
Section	**Changes**
8. Precision	Update of the precision data (limit of r and limit of R) after the performance of the CLEN 2nd proficiency test on Completely Denatured Alcohol

Determination of Ethanol in Alcoholic Products by GC-FID
(Gas Chromatography - Flame Ionisation Detection)

1. Scope

The method is suitable to determine ethanol in Completely Denatured Alcohol (CDA) required for calculation of the content of denaturing substances as specified in Regulation (EC) 3199/93 of 22 November 1993 and its amendments, concerning the mutual recognition procedures for the complete denaturing of alcohol (CDA) for the purpose of exemption from excise duty.

In addition, this method is also suitable for some alcoholic beverages such as vodka, rum, whisky, brandy, fruit distillates as well as fraudulent distillates containing denaturants. The method can be used also for determination of ethyl alcohol in commercial mixtures such as antifreeze and screen-wash preparations.

2. Principle

Ethanol is separated from other volatile substances (i.e. methanol, ethyl acetate or denaturants such as IPA and MEK) using capillary column gas chromatography and is detected by FID. The ethanol concentration is determined against a calibration curve using an internal standard.

3. Reagents and materials

3.1. Chemicals used and their associated safety phrases

- Ethanol (ethyl alcohol) ; CAS 64-17-1; purity 99.8 %.
- Methanol (methyl alcohol);CAS 67-56-1; purity 99.8 %.
- 1,4-dioxane; CAS 123-91-1; p.a. grade;
  1,4-dioxane is used as internal standard. Alternatively, any other substance chromatographically sufficiently separated from all other components and not present in the sample can be used as an internal standard.

3.2. Gases and other consumables

- Gases: helium purity min. 5.0; hydrogen purity min. 4.6; synthetic air free of hydrocarbons
- Automatic pipette: 0 - 10 ml.
- Chromatographic capillary column DB624 (J&W) length 30 m, inner diameter 0.32 mm and thickness of stationary phase 1.8 µm. *Alternative GC capillary columns to the recommended DB624 (30 m x 0.32 mm x 1.8 µm) can be used if a good separation of ethyl alcohol from possible interferences can be achieved. The alternative columns include e.g. RXI-624 SILMS, TRB 624, RX-1, DB-1, HP-5 and other capillaries based on non-polar stationary phases.*
- Chromatographic syringe 10 µl, 500 µl, 1000 µl

- Glass vials 2 ml and 20 ml with cap and septum
- Tips for automatic pipette
- Tool for closing and opening of glass vials 2 ml and 20 ml

The chemicals, gases, and consumables mentioned here may be replaced by those having equivalent specifications.

4. Apparatus

4.1. Analytical balance with precision of 0.1 mg.

4.2. An electronic densimeter.

4.3. A gas chromatograph (GC) with split/split less injector, flame ionization detector, auto sampler and PC for control of the GC and data processing.

5. Procedure

5.1. Preparation of calibration solutions

Solutions for calibration points 1-5 are prepared as follows: an empty 20 ml glass vial (including septum and cap) is weighed and its weight (m_s) is recorded. The balance is then tarred. 15 ml of methanol is added to the 20 ml glass vial. The vial is closed by cap and weighed (the weight of methanol m_m is recorded). The balance is tarred. Then 0.5 ml of internal standard (e.g. 1,4-dioxane) is added through a septum using a syringe, the content of the vial is mixed and weighed (the weight of internal standard m_i is recorded). The balance is tarred. Finally 10, 100, 300, 700 or 1000 µl of ethyl alcohol (for calibration standards 1 to 5, respectively) are added to the vial through the septum using a syringe. The content of the vial is mixed and weighed (the weight of ethyl alcohol m_a is recorded). The balance is tarred and the vial is removed from the balance. Final weight reading m_f is recorded (as positive value). The maximum acceptable difference between m_f and the sum of all the weights is 0.5 mg (see **Equation 1**). When the difference is higher, the preparation of that particular calibration solution must be repeated. Each calibration solution is mixed, then opened and 2 ml of the calibration solution is transferred to a 2 ml glass vial. The 2 ml glass vial is closed and stored in the refrigerator until the GC analysis is completed.

Note: Depending on final ethanol concentration in the sample the calibration range can be modified providing the quality control conditions described below are met.

5.2. Sample preparation

Solid impurities in sample must be filtrated.
Sample preparation is carried out exactly as described above, however, instead of ethanol, 1000 µl of the sample is added and the weight m_{vz} is recorded. Every sample is prepared in duplicate.

5.3. Chromatographic conditions

With regard to the operation of the GC, the analytical sequence is programmed in the GC software (e.g. sample number, sample position in auto sampler, analytical method, amount of the internal standard, dilution factor).

The chromatographic conditions to be used with the GC capillary column DB624 (30 x 0.32 mm x 1.8 µm) are as follows: inject volume: 1 µl, split approx. 120 ml/min., injector temperature 230 °C, detector FID temperature 230 °C, temperature gradient: 45 °C (4 min) / 10 °C/min / 90 °C (0 min) / 20 °C/min / 200 °C (2 min), carrier gas helium velocity (32 cm/s).

If a different type of GC column is used or if the ethyl alcohol peak co-elutes with the peak of another substance (i.e. separation of ethanol peak is less than 0.75 %) or if the shape of the ethyl alcohol peak is not obvious (broad peak, tailing peak, etc.), the GC temperature programme may be modified.

Between each sample injection, the auto-sampler must be programmed to thoroughly rinse the injection syringe using methanol to prevent any possible cross-contamination.

The chromatographic conditions can be modified for other columns used to achieve a good separation of ethyl alcohol and internal standard from possible interferences.

5.4. Quality control

Data for calibration curve construction are obtained by the measurement of 5 calibration solutions. The peak of the internal standard (this peak is defined as reference) and the ethyl alcohol peak are identified. A first order curve (line) is constructed from the points obtained by combination of the area of ethyl alcohol peak and its corresponding concentration. The calibration curve does not include the point [0; 0]. The calibration curve must have a correlation coefficient higher than 0.999. If not, the calibration must be repeated. The maximum acceptable intercept of the calibration curve (line) is |0.01| (absolute value). In case of a higher intercept, it is necessary to find the source of this problem and to repeat the calibration.

A quality control (QC-80) solution of approx. 80 % ethyl alcohol is prepared. The correctness of the calibration is checked by analysing the QC-80 solution immediately after the calibration. If the result for the QC-80 standard is outside the limits given for this standard (±0.4 % mass) the calibration must be repeated or at least re-evaluated. The method is checked by controlling the QC-80 standard at the beginning and at the end of a sample sequence (first and last vials contain QC-80). If the sequence contains more than 10 vials, this control standard is placed into the sequence after each 10 vials. The sample results are accepted only if the results for the QC-80 standard are within the limits given for this standard. If the result for the QC-80 standard is outside the limits given for this standard, it is not possible to accept the results for the samples placed before and after this standard. These samples must be prepared and measured again.

Note: Depending on samples different quality control solutions can be used having ethanol concentration close to the sample analysed e.g. QC-35 for distillates or QC-90 for denaturized alcohol.

Each sample is prepared in duplicate: if the difference between the first and the second sample result is higher than the repeatability limit, the sample must be prepared and measured in duplicate again.

6. Calculations

The concentrations of ethyl alcohol as well as the internal standard are calculated for each calibration solution using the **Equations 2** and **3**. The ethanol content in the sample is calculated using the GC software and is obtained from the calibration curve (evaluation is done using the area of the peaks) and multiplied by sample dilution factor (using **Equations 4** and **5**).

Equation 1 $m_f - (m_s + m_m + m_i + m_e) \leq 0.5$ [mg]

Equation 2 $\mathrm{C_{EtOH}} = w_v \times \frac{0.78924}{\rho_{EtOH}} \times \frac{m_a}{m_m + m_i + m_a}$ [% mass]

Equation 3 $C_i = 100 \times \frac{m_i}{m_m + m_i + m_a}$ [% mass]

Equation 4 $R = \frac{m_m + m_i + m_{VZ}}{m_{VZ}}$ [-]

Equation 5 $w_{EtOH} = R \times w'_{EtOH}$ [% mass]

Equation 6 $w_v = \rho_{VZ} \times \frac{w_{EtOH}}{0.78924}$ [% vol.]

ρ_{EtOH}	Density of calibration standard by 20 °C in g.cm^{-3}
ρ_{VZ}	Density of the sample by 20 °C in g.cm^{-3}
0.78924	Density of ethyl alcohol by 20 °C in g.cm^{-3}
C_I	Concentration of the internal standard in calibration solution in % mass
C_{EtOH}	Concentration of ethyl alcohol in calibration solution in % mass
m_a	Weight of the calibration standard
m_i	Weight of the internal standard in g
m_m	Weight of methanol in g
m_f	Weight of full 20 ml vial (with cap, septum, methanol, internal standard and calibration standard/sample) in g
m_s	Weight of empty 20 ml vial with cap and septum in g
m_{vz}	Weight of the sample in g
R	Dilution factor
w'_{EtOH}	Concentration of ethyl alcohol in the sample solution in % mass (reading from the calibration curve)
w_{EtOH}	Concentration of ethyl alcohol in sample in % mass
w_v	Concentration of ethyl alcohol in % volume

Duplicate measurements are rounded to 0.01 % mass.
The final result is the arithmetic mean of duplicate measurements for each sample, rounded to 0.1 % mass.
It is possible to recalculate the result from % mass to % vol. using the **Equation 6**.

7. Expression of results

Result are expressed with maximum 3 significant figures, with maximum one decimal place, in % vol.

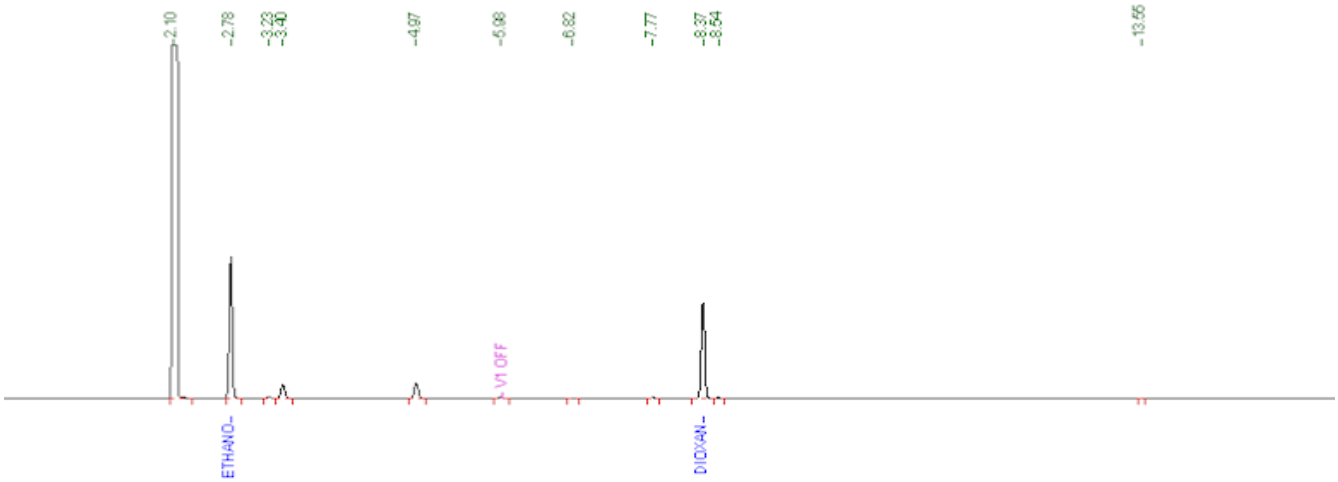
8. Precision

Precision data (limits of r and R) obtained from the CLEN 1st and 2nd proficiency test on Completely Denatured Alcohol, performed in 2019 (by 41 laboratories on 3 samples; final report issued 4 September 2019) and in 2021 (by 49 laboratories on 4 samples; final report issued 14 April 2022):

Ethanol content (robust mean), % vol.	For matrices with a range of ethanol between 22.7 and 94.6 %vol.
Repeatability, *% vol.*	**0.6**
Reproducibility *% vol.*	**2.5**

Annex – examples of chromatograms

Example of the typical chromatogram of a sample containing ethanol:



Typical chromatogram of a formulation including the internal standard (1,4-dioxane) and a series of other potential denaturants.