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**Petroleum products — Biodiesel —
Determination of total esters content
by gas chromatography**

*Produits pétroliers — Biodiesel — Dosage de la teneur en esters
totale par chromatographie en phase gazeuse*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 28, *Petroleum products and related products of synthetic or biological origin*, Subcommittee SC 7, *Liquid biofuels*.

Petroleum products — Biodiesel — Determination of total esters content by gas chromatography

WARNING — The use of this Technical Specification might involve the usage of dangerous materials and equipment. It is the responsibility of the user to establish the appropriate security, health and environmental practices, and to determine the applicability of regulatory limitations before their use.

1 Scope

This Technical Specification establishes a method for determining the total methyl ester content in fatty acid methyl ester (FAME) by gas chromatography and using an external standard. The method is suitable for biodiesel which contains esters between C6 and C26. This method allows verifying that the total ester content is greater than 96,5 % (*m/m*).

NOTE 1 The method also allows determination of the total ethyl ester content in FAEE, but precision for this has not been established.

This Technical Specification does not determine the linolenic nor the poly-unsaturated alkyl ester content. Alternative techniques, such as EN 14103[1] and EN 15779[2], respectively, are available for this.

NOTE 2 For the purposes of this Technical Specification, the term “% (*m/m*)” is used to represent the mass fraction, μ .

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3170, *Petroleum liquids — Manual sampling*

ISO 3171, *Petroleum liquids — Automatic pipeline sampling*

ISO 4259, *Petroleum products — Determination and application of precision data in relation to methods of test*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

biodiesel

fuel comprised of monoalkyl esters of fatty acids, derived from vegetable oils or animal fat

3.2

total esters

sum of concentration of all esters (C6 – C26)

4 Principle

A sample is analysed by gas chromatography using an external calibration method to quantify the esters present in biodiesel regardless of the raw material used in its production.

The quantification of biodiesel samples with C18 carbon chains is performed using an analytical curve prepared with methyl or ethyl octadecanoate. If there is presence of esters with carbon chain shorter than C₁₄, in addition methyl or ethyl dodecanoate is used for quantifying these esters in the sample.

It is recommended to consult the chromatograms presented in [Annex A](#) to facilitate the identification of different matrices of biodiesel.

5 Apparatus

5.1 Gas chromatograph equipped with flame ionization detector (FID), an on-column injector (or equivalent) and oven with temperature programming.

5.2 Data acquisition system, an electronic instrument to obtain and record the peak area in the chromatograms.

5.3 Column, fused silica capillary column, 30 m × 0,32 mm × 0,1 μm, with stationary phase 95 % dimethylpolysiloxane and 5 % phenyl-methylpolysiloxane for high temperature (up to 400 °C).

Any column with better or equivalent efficiency and selectivity may be used. Their usefulness should be observed by comparing the chromatogram obtained with chromatograms presented in [Annex A](#).

NOTE A retention gap of 0,53 mm of internal diameter can be used.

5.4 Automatic sampler.

5.5 Balance, with resolution of 0,1 mg.

5.6 Flask, with the capacity of 50 mL, with lid.

5.7 Micro syringe, with the capacity for 1,0 μl.

5.8 Pasteur pipettes.

5.9 Beaker of 50 ml.

6 Reagents and materials

6.1 *n*-heptane, (CAS¹) N° 142-82-5), minimum purity of 99 %.

6.2 Methyl dodecanoate (CAS¹) N° 111-82-0) or ethyl dodecanoate (CAS¹) N° 106-33-2), minimum purity of 99 %.

6.3 Methyl octadecanoate (CAS¹) N° 112-61-8) or ethyl octadecanoate (CAS¹) N° 111-61-5), minimum purity of 99 %.

6.4 Carrier gas, hydrogen or helium, minimum purity of 99,999 %, as carrier gas (for detector gas a FID suitable grade is allowed).

6.5 Nitrogen, minimum purity of 99,999 %, grade suitable for FID.

1) Represents the register of chemical substances catalogued in the CAS system. CAS numbers have no chemical meaning, these are numbers designated in sequential order for each substance added to the system.

6.6 Synthetic air, minimum purity of 99,999 %, grade suitable for FID.

7 Sampling

Unless otherwise specified, obtain representative samples for analysis in accordance with the procedures given in ISO 3170, ISO 3171 or an equivalent International Standard.

8 Procedure

8.1 Preparation of the apparatus

8.1.1 Install the column according to the instructions of the manufacturer.

8.1.2 Establish a flow of about 3,0 ml/min of helium (pressure around 180 kPa and average linear velocity around 0,54 m/s and) or hydrogen (pressure around 105 kPa and average linear velocity around 0,70 m/s).

8.1.3 Adjust the following typical operation conditions on the gas chromatograph:

a) programming the oven temperature:

Heating rate °C/min	Temperature °C	Holding time min
—	50	1
15	180	0
7	230	0
20	380	10

b) carrier gas: helium or hydrogen;

c) detector temperature: 380 °C;

d) injector temperature: oven tracking;

e) nitrogen flow for the detector (*make-up gas*): 30 ml/min;

f) hydrogen flow for the detector: 35 ml/min;

g) synthetic air flow for the detector: 350 ml/min;

h) volume to be injected: 0,5 µl;

i) approximate time of analysis: 35 min.

NOTE The detector flows recommended by the manufacturer can also be used.

8.1.4 Evaluate the stability of the baseline running a blank.

8.1.5 After the system stabilizes baseline subtraction or electronic compensation of the signal, following the procedures inside the equipment's manual may be applied to eliminate the deviation of the baseline due to the temperature programming of the oven.

8.2 Preparation of the calibration curve

8.2.1 For biodiesel samples containing mainly fatty acids with C₁₆ and C₁₈ carbon chains, a calibration curve shall be prepared with methyl or ethyl octadecanoate.

8.2.2 In samples that contain fatty acids with carbon chains less than C₁₄, two calibration curves are required; one using methyl or ethyl dodecanoate to quantify these esters and another using methyl or ethyl octadecanoate to quantify all the others esters.

8.2.3 Prepare a minimum of six standard solutions for any calibration curve, each one corresponding to a point in the curve.

An example of preparation procedure for six points of standard solutions of methyl or ethyl octadecanoate is found in [Annex B](#).

8.2.4 Inject 0,5 µl of the standard solution, at least two times.

Identify the peaks according to the chromatograms in [Annex A](#) and obtain the area of each component.

NOTE Depending on the construction of different instruments, there is a risk that the linear range of the FID is exceeded. Check the peak shape to secure that the detector/amplifier has not become overloaded/saturated.

8.2.5 Obtain the calibration curve of each component of the solution by plotting the area in (y) axis versus concentration in mass percentage in (x) axis.

A calibration curve is acceptable if its regression coefficient is >0,999.

8.3 Sample preparation

8.3.1 Add approximately 0,1 g with an accuracy of 0,1 mg of sample to a 50 ml beaker ([5.9](#)) and record the mass.

8.3.2 Dilute with 26,8 g of *n*-heptane and determine the total mass of solution.

Homogenize and transfer to the vial of automatic sampler.

8.4 Procedure

8.4.1 Inject 0,5 µl of sample solution (see [8.3](#)) at least two times.

8.4.2 Identify and integrate all the peaks of esters of the sample from C₆ up to C₂₆.

For guidance, see the chromatograms of [Annex A](#).

The identification of peaks relating to esters may be done using a standard chromatogram obtained by gas chromatography/mass spectrometry (GC/MS). In that case, GC/MS and GC/FID configurations shall be equivalent.

9 Expression of results

Calculate the total ester content with Formula (1):

$$C = \frac{A - b}{a \times m_a} \times m_t \quad (1)$$

where

C is the mass percentage [% (m/m)] of total ester content of the sample;

A is the sum of peak areas of the identified esters of the sample;

a is the slope (angular coefficient) of the calibration curve;

b is the intercept obtained for the calibration curve

m_a is the mass of sample used in 8.3 in grams (g);

m_t is the total mass (mass of sample + mass of *n*-heptane), in grams (g)

Express the results in % (m/m) to the nearest 0,1 % (m/m).

10 Precision

10.1 General

The precision given in 10.2 and 10.3 was determined by statistical examination of interlaboratory test results in accordance with ISO 4259.

10.2 Repeatability, r

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material, would in the long run, in the normal and correct operation of the test method, exceed the values calculated according to Formula (2) only in one case in twenty.

$$r = -53,404 + 0,595\ 6 \cdot X [\%] \quad (2)$$

where

X represents the mean of the two results expressed in % (m/m).

10.3 Reproducibility, R

The difference between two single and independent test results, obtained by different operators working in different laboratories on identical test material, would in the long run, in the normal and correct operation of the test method, exceed the values calculated according to the Formula (3) only in one case in twenty.

$$R = 17,461\ 2 + -0,128\ 9 \cdot X [\%] \quad (3)$$

where

X represents the mean of the two results expressed in % (m/m).

11 Test report

The test report shall specify the following:

- a) the reference to this Technical Specification, i.e. ISO/TS 17307:—;
- b) the type and complete identification of the product tested;
- c) the used method of sampling;
- d) the results of the test (see [Clause 9](#));
- e) all operating details not specified in this Technical Specification, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- f) the date of the test.

Annex A (informative)

Examples of chromatograms

This Annex shows examples of chromatograms that should be used as reference to compare with own chromatograms obtained through the laboratory set-up. All peaks in the chromatograms that are not numbered have not been identified as ester peaks. One should, however, be aware of the possibility that peaks of esters may overlap with other peaks, for instance from glycerides.

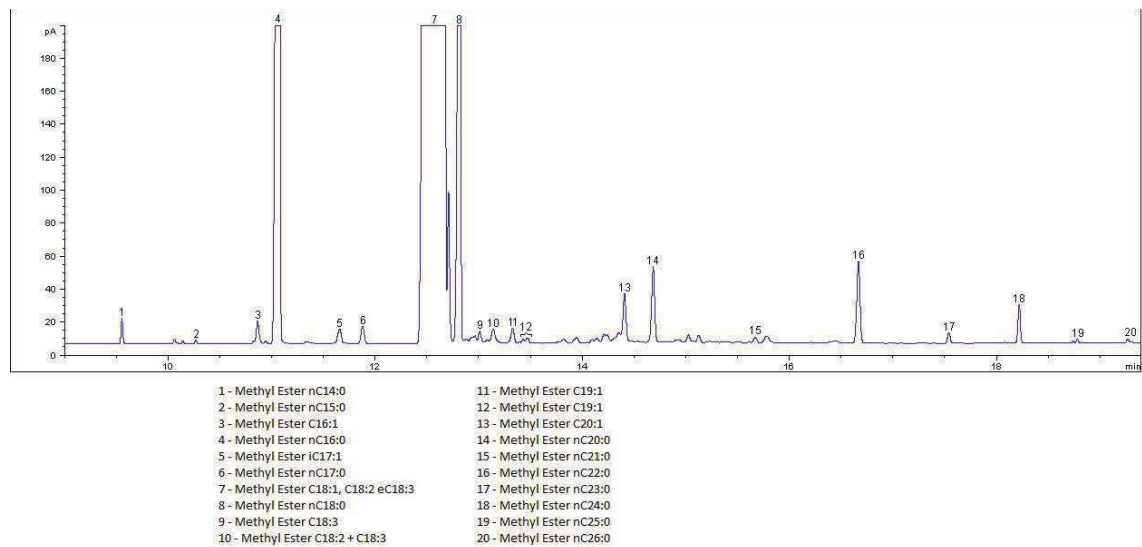


Figure A.1 — Soy biodiesel sample

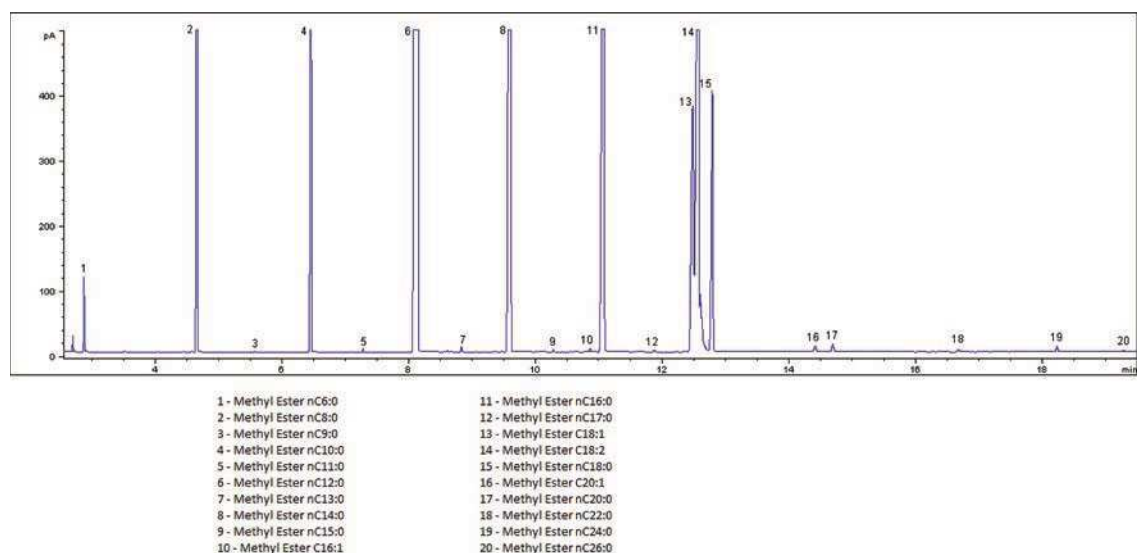


Figure A.2 — Coconut biodiesel sample

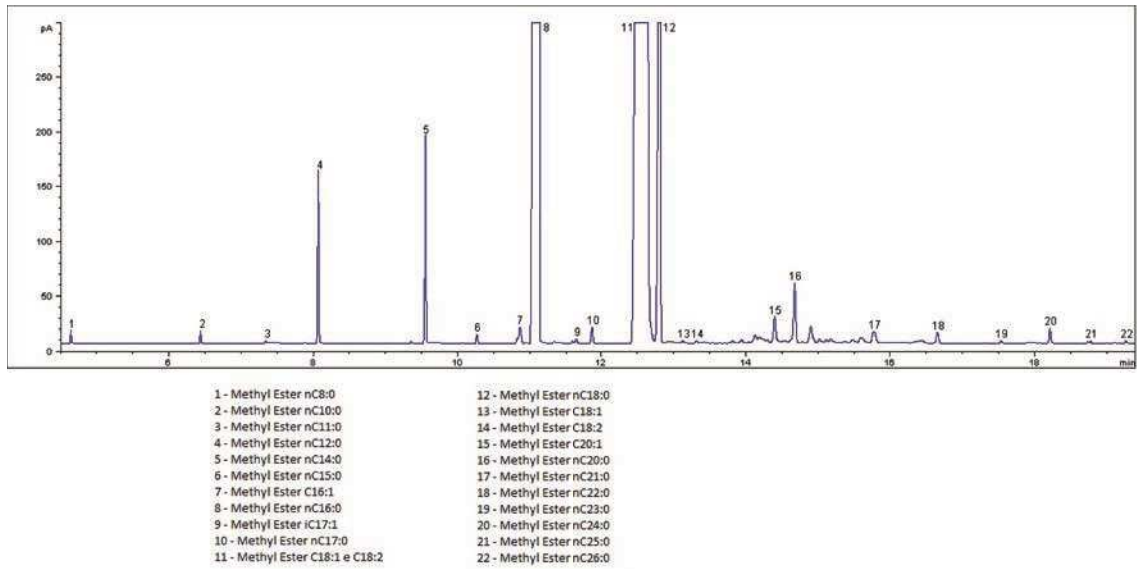


Figure A.3 — Palm biodiesel sample

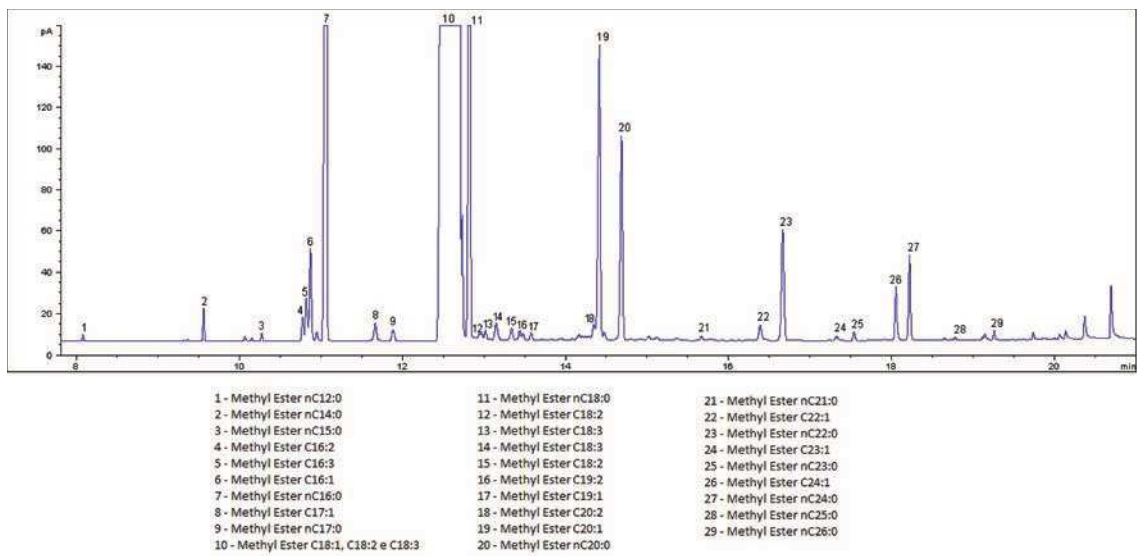


Figure A.4 — Rapeseed biodiesel sample

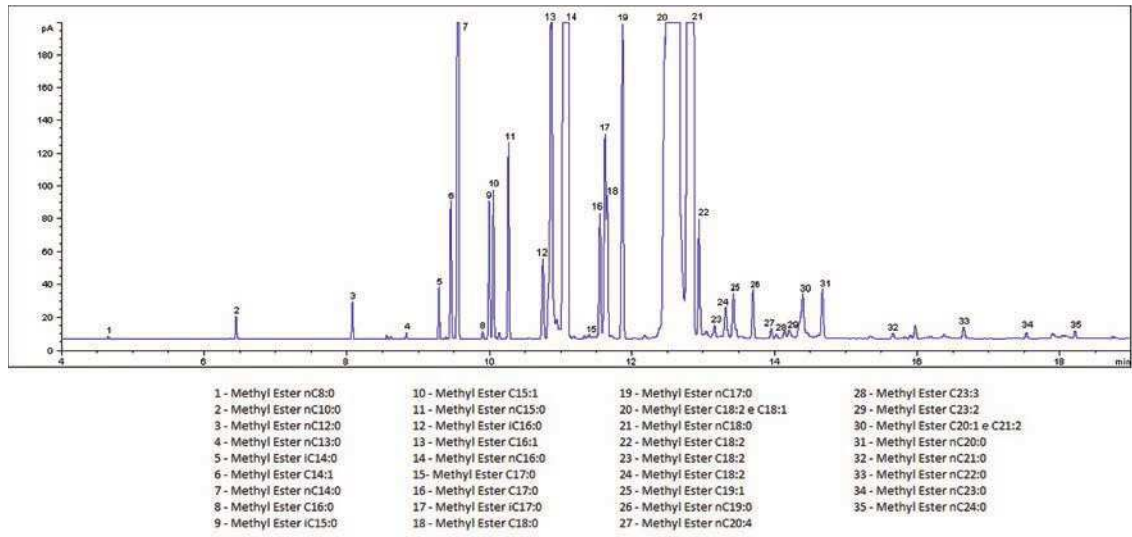


Figure A.5 — Animal fat biodiesel sample

Annex B (informative)

Preparation of standard solutions

Prepare a standard solution of methyl (or ethyl) octadecanoate in mass corresponding to approximately 40 mL of *n*-heptane according to [Table B.1](#).

This procedure also applies to preparation of solution with methyl (or ethyl) dodecanoate.

Table B.1 — Preparation of standard solutions

Level	Compound mass g	Total mass (compound + <i>n</i> -heptane) g	Compound concentration ^a %
1	0,001 0	26,801	0,003 7
2	0,005 0	26,805	0,018 7
3	0,010 0	26,810	0,037 3
4	0,040 0	26,840	0,149 0
5	0,090 0	26,890	0,334 7
6	0,099 0	26,899	0,368 0

^a Compound concentration (m/m) in sample corresponding to: 1 %, 5 %, 10 %, 40 %, 90 % and 99 %.

Bibliography

- [1] EN 14103, *Fat and oil derivatives — Fatty Acid Methyl Esters (FAME) — Determination of ester and linolenic acid methyl ester contents*
- [2] EN 15779, *Petroleum products and fat and oil derivatives — Fatty acid methyl esters (FAME) for diesel engines — Determination of polyunsaturated (≥ 4 double bonds) fatty acid methyl esters (PUFA) by gas chromatography*

