
**Petroleum products — Biodiesel
— Determination of free and
total glycerin and mono-, di- and
tracylglycerols by gas chromatography**

*Produits pétroliers — Biogazole — Détermination de glycérine
libre et totale et des mono-, di- et tracylglycerols avec
chromatographie 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).

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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*.

Introduction

This Technical Specification establishes a method for quantitative determination of free glycerol, mono-, di-, triacylglycerols and total glycerol in fatty acid methyl esters (biodiesel) by gas chromatography. High concentrations of these components can contribute to formation of deposits on the pistons and valves of diesel cycle engines. Additionally, they can cause problems during storage and in the engine's fuel injection system.

Alternative methods for similar determinations exist in ASTM D6584^[2] and EN 14105^[3] which are tailor made to regional quality specification needs. This Technical Specification describes an alternative technique using more easily available internal standards, instrumentation that can also measure esters and a procedure applicable to short chain fatty acid esters, such as those from palm kernel and coconut oil. This Technical Specification thus provides a wider usage with similar or worse precision as other techniques.

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Petroleum products — Biodiesel — Determination of free and total glycerin and mono-, di- and triacylglycerols by gas chromatography

WARNING — The use of this Technical Specification may 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 methodology for quantitative determination of free glycerol, mono-, di-, triacylglycerols and total glycerol by gas chromatography in biodiesel produced from any raw material including coconut or palm oil and animal fat. It is not applicable for biodiesel from castor oil.

In most actual cases, biodiesel is based on fatty acid methyl esters (FAME). These have also been used during the precision study for this test method. There is no indication that the methodology does not apply to other ester types, but the precision has not been determined nor compared.

NOTE 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*

3 Terms and definitions

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

3.1

biodiesel

fuel comprised of mono-alkyl esters of fatty acids, derived from vegetable oils or animal fats

3.2

bonded glycerol

glycerol portion of the mono-, di-, and triacylglycerols molecules

3.3

total glycerol

sum of free glycerol and bonded glycerol

3.4

monoacylglycerols

sum of monostearin, monopalmitin, monoolein, monolinolein, concentrations and/or other monoacylglycerols present in the biodiesel

3.5

diacylglycerols

sum of diolein, dilinolein concentrations and/or other diacylglycerols present in the biodiesel

3.6

triacylglycerols

sum of triolein, trilinolein concentrations and/or other triacylglycerols present in the biodiesel

3.7

silylation

reaction to substitute the active hydrogen present in the mono- and diacylglycerol molecules to obtain more volatile and stable compounds

4 Principle

A sample is injected into a gas chromatograph after silylation with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). The identification of the components in the sample is done by comparing the retention times of four reference materials (glycerol, monoolein, diolein and triolein). The quantification is done using calibration curves with internal standardization. For quantifying the glycerin and the acylglycerols, ethylene glycol and tricaprins are used as internal standard, respectively. The total glycerol is obtained from the sum of free and bonded glycerol concentrations.

5 Apparatus

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

NOTE 1 Any column with better or equivalent efficiency and selectivity can be used. Their usefulness can be observed by comparing the chromatogram obtained with chromatograms presented in [Annex A](#).

NOTE 2 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 Volumetric flasks of 50 ml, 25 ml and 10 ml

5.7 Appropriate vials for automatic sampler, screw top vials can lead to sample evaporation.

5.8 Flask, with a capacity of 10 ml, with polytetrafluoroethylene (PTFE) faced septa.

5.9 Microlitre syringes, with a capacity of 5 µl for sample injection.

5.10 Microlitre syringes or micropipette, with a capacity of 100 µl and 250 µl for the preparation of the solutions.

5.11 Pasteur pipettes.

5.12 Volumetric pipettes, graduated, with a capacity of 10 ml and 20 ml.

6 Reagents and materials

6.1 n-heptane, 99,0 % minimum purity.

6.2 Pyridine (dried), 99,0 % minimum purity, with a maximum water content of 0,1 %.

It is recommended that the pyridine be stored with a molecular sieve 5A, 4/8 mesh. Its conditioning should be undertaken in a lab oven, at 350 °C throughout the night. Allow cooling down in a desiccant, without silica.

6.3 1-Glycerolmonooctadecenoate (glycerol monooleate or monoolein) (CAS¹⁾ No. 111-03-5), 99,0 % minimum purity.

6.4 1,3 Glycerol dioctadecenoate (glycerol dioleate or diolein) (CAS No. 2465-32-9), 99,0 % minimum purity.

6.5 Glycerol trioctadecenoate (glycerol trioleate or triolein) (CAS No. 122-32-7), 99,0 % minimum purity.

6.6 Glycerol (CAS N° 56-81-5), 99,5 % minimum purity.

6.7 Ethylene glycol (CAS No. 107-21-1), 99,0 % minimum purity.

6.8 Tricaprin (CAS N° 621-71-6), 99,0 % minimum purity.

6.9 N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), reagent grade.

IMPORTANT — For silylation purposes, interaction with water shall be prevented.

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

6.11 Nitrogen, grade suitable for FID.

6.12 Synthetic air, grade suitable for FID.

7 Preparation of the apparatus

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

7.2 Establish a carrier gas flow of around 3,0 ml/min in the column (pressure of 180 kPa and average linear velocity of around 0,54 m/s if helium is used or 105 kPa and 0,70 m/s to hydrogen).

7.3 Adjust the following typical operating conditions on the gas chromatograph:

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.

a) oven temperature programming:

Oven program 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 (make-up gas): 30 ml/min;

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

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

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

h) volume injected: 1,0 µl;

i) run time: 35 min.

7.4 Evaluate the stability of the baseline running a blank.

7.5 After system stabilization, baseline subtraction or electronic compensation following the procedures described in the equipment's manual can be applied to eliminate the deviation of the baseline due to the temperature programming of the oven. Care should be taken not to lose the raw data if required for good laboratory practices.

8 Sampling

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

9 Preparation of the standard solutions

9.1 Preparation of the stock solutions

In a flask with adequate capacity, prepare stock solutions for each substance according to [Table 1](#), recording the respective masses.

Store and keep the solutions in the refrigerator when not in use.

NOTE Under these conditions, the stock solutions can be stored for one month.

Table 1 — Preparation of the stock solutions

Compound	Approximate mass mg	Pyridine mass G
Glycerin	25	49,0
Monoolein	50	9,80
Diolein	50	9,80
Triolein	50	9,80
Ethylene glycol (internal standard 1)	25	24,5
Tricaprin (internal standard 2)	80	9,80

Calculate the concentrations of the stock solutions using Formula (1):

$$C_{ss} = \frac{m_{ss}}{m_T} \times 100 \quad (1)$$

where

C_{ss} is the concentrations of the compounds in the stock solutions in % (m/m);

m_{ss} is the mass of the compounds used in the preparation of the stock solutions, with a precision of 0,1 mg;

m_T is the total mass ($m_{\text{compound}} + m_{\text{pyridine}}$), with a precision of 0,1 mg.

9.2 Preparation of the calibration curve

9.2.1 In a flask with a capacity of 10 ml (5.8), prepare the standard solution one by adding the stock solutions according to the indications on Table 2. Record the masses of each stock solution added.

9.2.2 Repeat the same procedure for the other standard solutions 2, 3, 4 and 5 from Table 2.

9.2.3 Add to each of the five standard solutions 100 µl of MSTFA. Close the flask and shake vigorously. Let it react for at least 20 min at room temperature.

9.2.4 After the reaction time, add approximately 8 ml of n-heptane and shake, transfer a portion to an automatic sampler vial and seal.

9.2.5 Inject 1,0 µl of each solution, at least twice. Identify the peaks according to their retention times; an elution order is presented in Figure A.1.

Table 2 — Preparation of the standard solutions

Standard solution	1	2	3	4	5
Stock solution of glycerin (µl)	10	30	50	70	100
Stock solution of monoolein (µl)	20	50	100	150	200
Stock solution of diolein (µl)	10	20	40	70	100
Stock solution of triolein (µl)	10	20	40	70	100
Stock solution of ethylene glycol (µl)	100	100	100	100	100
Stock solution of tricaprinn (µl)	100	100	100	100	100

9.2.6 Use Formula (2) to calculate the mass of all the compounds present in the standard solutions which will be used in calibration curves.

$$m_c = \frac{C_S}{100} \times m_A \quad (2)$$

where

m_C is the mass of the compounds used in the preparation of the standard solutions, with a precision of 0,1 mg;

C_S is the concentrations of the compounds in the standard solutions in % (m/m);

m_A is the mass corresponding to the volumes of each compound added to standard solutions, with a precision of 0,1 mg.

9.2.7 Obtain the areas corresponding to each component. Calculate the area ratio (y) and the mass ratio (x) using Formulae (3) and (4):

$$y = \frac{A_C}{A_{IS}} \quad (3)$$

where

A_C is the area of the compound;

A_{IS} is the area of the internal standard.

$$x = \frac{m_C}{m_{IS}} \quad (4)$$

where

m_C is the mass of the compound, with a precision of 0,1 mg;

m_{IS} is the mass of the internal standard, with a precision of 0,1 mg.

9.2.8 Prepare the calibration curve for each reference component by plotting the area ratio (y) versus mass ratio (x).

After acquiring the areas correspondent to each peak, use the angular and linear coefficients of the formula obtained for each compound of reference (9.2.7):

$$y = a \cdot x + b \quad (5)$$

where

a is the slope (angular coefficient) obtained for the calibration curve;

b is the intercept (linear coefficient) obtained for the calibration curve.

9.2.9 Check whether the regression coefficient is at least 0,99, otherwise recalibrate.

9.3 Preparation of the sample

9.3.1 Add 100 mg of the sample in a flask with a capacity of 10 ml and record the mass.

9.3.2 Add 100 µl of each internal standard stock solution (see 9.1) and record the masses, m_{ISS} , with a precision of 0,1 mg.

9.3.3 Add 100 µl of MSTFA. Close the flask and shake vigorously. Allow to react for 20 min at room temperature.

9.3.4 After the reaction time, add approximately 8 ml of n-heptane to the flask and shake vigorously. Transfer a portion to an automatic sample vial and seal.

9.4 Procedure

9.4.1 Inject 1,0 µL of the solution of sample. Identify the components of interest using the chromatograms presented in Annex A. Carry out the determination in duplicate.

To eliminate any doubts on the identification of the peaks of mono-, di- and triacylglycerols, since these compounds can vary according to the type of raw material used to produce the biodiesel, the use of relative retention time is recommended. Table 3 presents an example from soy biodiesel analysis in Figure A.2.

9.4.2 The use of a reference standard of monoacylglycerols may be applied for identification of individual monoacylglycerols in B100. The monoacylglycerol mixtures, containing monoolein, monostearin and monopalmitin should be prepared prior to analysis. Weigh 100 mg of each monoacylglycerol and dissolve in 10 ml of pyridine.

The mixture also can be purchased commercially.

Then, transfer 100 µl of the monoacylglycerol mixture and 100 µl of MSTFA into a 10 ml vial. Close the vial and shake it. Allow the vial to stand for at least 20 min at room temperature. Add about 8 ml of heptane to the vial and shake. Avoid contact with moisture.

Table 3 — Example of relative retention times obtained from soy biodiesel analysis

Compound	T_R/T_{RP11}	T_R/T_{RP12}
Glycerin	1,82	—
Ethylene glycol	1,00	—
Monopalmitin	—	0,84
Monoolein + Monolinolein	—	0,89
Monolinoleic + Monolinolenic	—	
Monostearin	—	0,90
Tricaprin	—	1,00
Diacylglycerol	—	1,05
Diacylglycerol	—	1,07
Diacylglycerol	—	1,09
Triacylglycerol	—	1,17
Triacylglycerol	—	1,20
Triacylglycerol	—	1,24
T_R Retention time of the compound.		
T_{RP11} Retention time of the internal standard 1 (Ethylene glycol).		
T_{RP12} Retention time of the internal standard 2 (Tricaprin).		

10 Calculation and expression of results

10.1 From the integration of the glycerol, ethylene glycol (internal standard 1), mono-, di-, triacylglycerols and tricaprin (internal standard 2) peaks, obtain the area of each compound.

It is recommended to integrate jointly the two major diacylglycerol peaks, on account of an insufficient resolution which can induce quantification errors if the two peaks are integrated separately. The presence of a double peak at the level of the glycerol retention time leads to verification of the silylation stage, which is then probably incomplete (i.e. presence of water in the samples).

10.2 Obtain the mass of internal standard added to the sample using Formula (6):

$$m_c = \frac{C_{ISS} \times m_{ISS}}{100} \quad (6)$$

where

m_{IS} is the mass of the internal standard in the sample, with a precision of 0,1 mg;

C_{ISS} is the concentration of the internal standard in the stock solution, in % (m/m);

m_{ISS} is the mass of the internal standard in the stock solution added in the sample (9.3.2).

10.3 Glycerol

The results are determined as follows:

$$G = \left[\frac{m_{PI1}}{a_g} \right] \left[\left(\frac{A_g}{A_{PI1}} \right) - b_g \right] \left[\frac{100}{m} \right] \quad (7)$$

where

G is the concentration of free glycerol in % (m/m);

A_g is the area of the glycerol;

A_{PI1} is the area of the ethylene glycol;

m_{PI1} is the mass of the ethylene glycol;

m is the mass of the sample;

a_g is the slope for the calibration curve of glycerol;

b_g is the intercept for the calibration curve of glycerol.

10.4 Acylglycerols (mono-, di- and triacylglycerols)

The results are determined as follows:

$$Glic = \left[\frac{m_{PI2}}{a_{glic}} \right] \left[\left(\frac{A_{glic}}{A_{PI2}} \right) - b_{glic} \right] \left[\frac{100}{m} \right] \quad (8)$$

where

$Glic$ is the concentration of mono-, di- or triacylglycerols in % (m/m);

A_{glic} is the sum of the areas peaks of the mono-, di- or triacylglycerols;

A_{PI2} is the area of the tricaprin;

m_{PI2} is the mass of the tricaprin;

m is the mass of the sample;

a_{glic} is slope for the calibration curve of mono-, di- or triacylglycerols;

b_{glic} is intercept for the calibration curve of mono-, di- or triacylglycerols.

10.5 Calculation of bonded glycerol

The results are determined as follows:

$$G_{\text{C}} = M + D + T \quad (9)$$

where

G_{C} is the concentration of bonded glycerol in % (m/m);

M is equal to $G_{\text{lm}} \times G_{\text{licmono}}$, % (m/m);

D is equal to $G_{\text{ld}} \times G_{\text{licdi}}$, % (m/m);

T is equal to $G_{\text{lt}} \times G_{\text{lictri}}$, % (m/m).

where

G_{licmono} is equal to 0,259 1;

G_{licdi} is equal to 0,148 8;

G_{lictri} is equal to 0,104 4.

The coefficients G_{licmono} , G_{licdi} and G_{lictri} utilized above are valid for calculations of bonded glycerol in biodiesel produced from typical vegetable oils such as soy, cotton or sunflower, canola, corn and animal fat. They are not valid for biodiesel produced from lauric oils or other special oils with a different average molecular weight. In [Annex B](#), it is indicated how to calculate the coefficients for other types of biodiesel.

10.6 Calculation of total glycerol

The results are determined as follows:

$$G_{\text{T}} = G_{\text{L}} + G_{\text{C}} \quad (10)$$

where

G_{T} is the total glycerol concentration in % (m/m).

10.7 The final concentration of each substance, in % (m/m), is obtained from an average between the values found for the individual injections of the sample.

10.8 Express the results in % (m/m) with two decimals.

11 Precision

11.1 General

The precision given in 11.2 and 11.3 was determined by statistical examination of inter-laboratory test results (see Annex C) in accordance with ISO 4259.[1]

NOTE The statistical examination is scheduled to be finalised halfway 2014.

11.2 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 the following formulae only in one case in 20.

Free glycerol

$$r = 7,732\ 5 + 0,007\ 7 \cdot X \left[mg/kg \right]$$

Total glycerol

$$r = 35,295 + 0,026\ 4 \cdot X \left[mg/kg \right]$$

Monoacylglycerols

$$r = 152,165\ 1 + 0,001\ 5 \cdot X \left[mg/kg \right]$$

Diacylglycerols

$$r = 62,546\ 8 + 0,036\ 8 \cdot X \left[mg/kg \right]$$

Triacylglycerols

$$r = 88,307\ 8 + 0,037\ 9 \cdot X \left[mg/kg \right] \quad (11)$$

where

X represents the mean of the two results expressed in mg/kg.

11.3 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 following formulae only in one case in 20.

Free glycerol

$$r = 53,163 + 0,234\ 1 \cdot X \left[mg/kg \right]$$

Total glycerol

$$r = 610,778\ 6 + 0,012\ 9 \cdot X \left[mg/kg \right]$$

Monoacylglycerols

$$r = 152,165\ 1 + 0,001\ 5 \cdot X \left[mg/kg \right]$$

Diacylglycerols

$$r = 200,788\ 9 + 0,296\ 3 \cdot X \left[\text{mg/kg} \right]$$

Triacylglycerols

$$r = 383,358\ 8 + 1,161\ 6 \cdot X \left[\text{mg/kg} \right] \quad (12)$$

where

X represents the mean of the two results expressed in mg/kg.

12 Test report

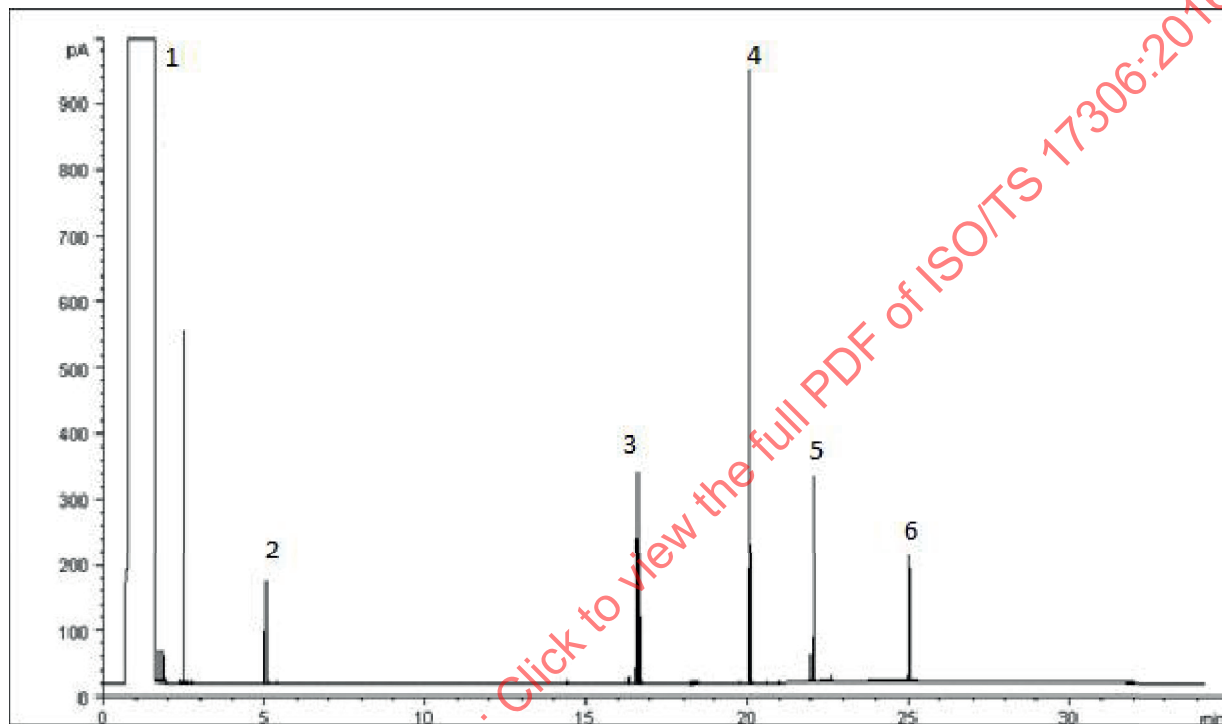
The test report shall specify the following:

- a) a reference to this Technical Specification, i.e. ISO/TS 17306:2015;
- b) the type and complete identification of the product tested;
- c) the used method of sampling;
- d) the results of the test (see [Clause 10](#));
- 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 (normative)

Check chromatograms

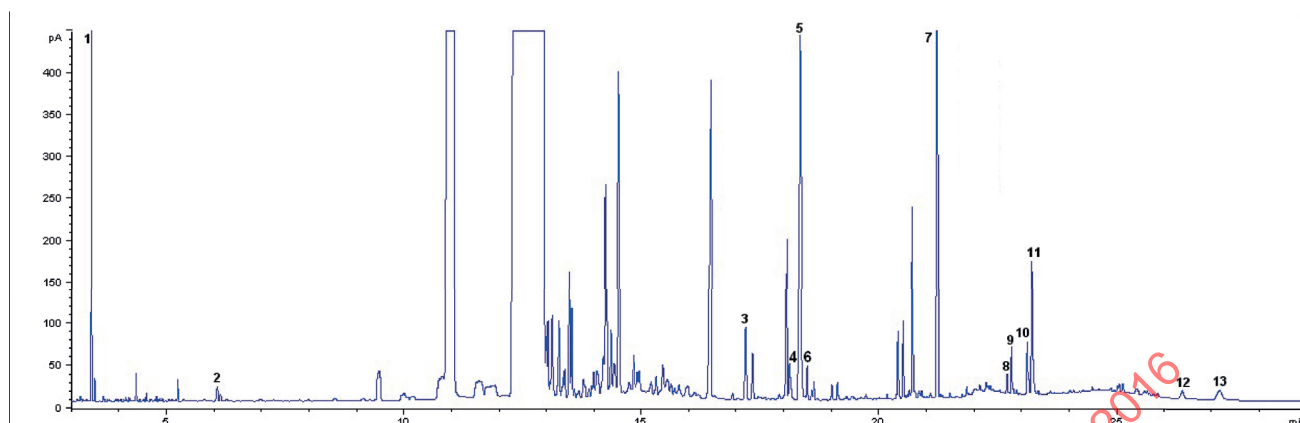
This Annex shows examples of chromatograms that should be used as reference to compare with own chromatograms obtained through the laboratory setup.



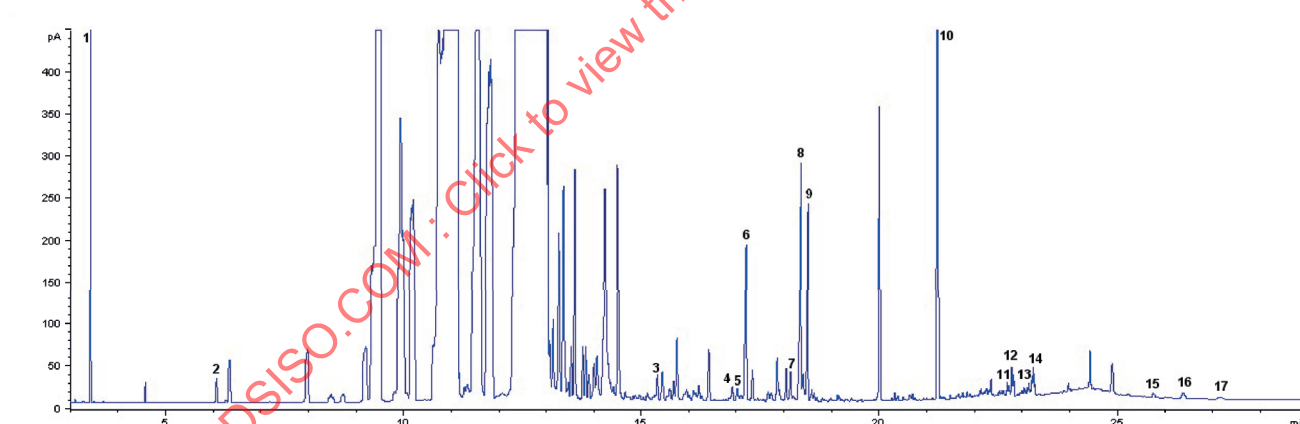
Key

- | | | | |
|---|-----------|---|-----------|
| 1 | solvent | 4 | tricaprin |
| 2 | glycerol | 5 | diolein |
| 3 | monoolein | 6 | triolein |

Figure A.1 — Chromatogram of a standard mixture

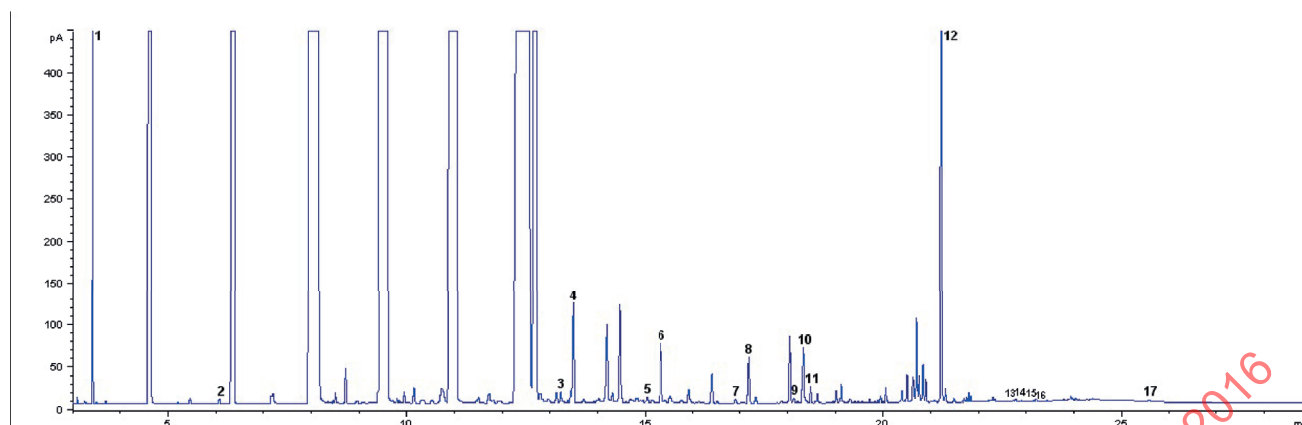
**Key**

- | | |
|--------------------|--------------------|
| 1 ethylene glycol | 8 diacylglycerol |
| 2 glycerol | 9 diacylglycerol |
| 3 monoacylglycerol | 10 diacylglycerol |
| 4 monoacylglycerol | 11 diacylglycerol |
| 5 monoacylglycerol | 12 triacylglycerol |
| 6 monoacylglycerol | 13 triacylglycerol |
| 7 tricaprin | |

Figure A.2 — Expanded chromatogram of soy biodiesel**Key**

- | | |
|--------------------|--------------------|
| 1 ethylene glycol | 10 tricapin |
| 2 glycerol | 11 diacylglycerol |
| 3 monoacylglycerol | 12 diacylglycerol |
| 4 monoacylglycerol | 13 diacylglycerol |
| 5 monoacylglycerol | 14 diacylglycerol |
| 6 monoacylglycerol | 15 triacylglycerol |
| 7 monoacylglycerol | 16 triacylglycerol |
| 8 monoacylglycerol | 17 diacylglycerol |
| 9 monoacylglycerol | |

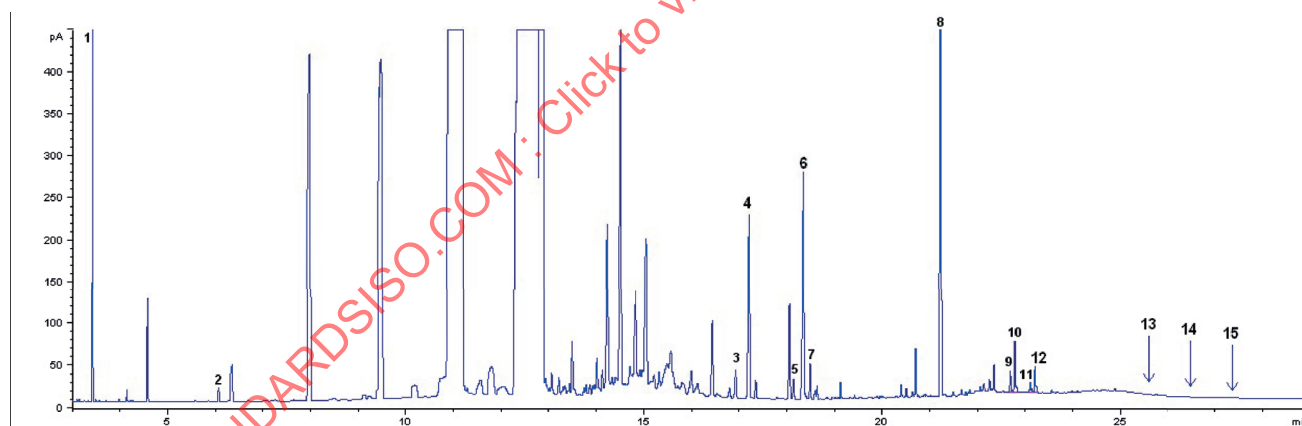
Figure A.3 — Expanded chromatogram of animal fat biodiesel



Key

- | | |
|--------------------|---------------------|
| 1 ethylene glycol | 10 monoacylglycerol |
| 2 glycerol | 11 monoacylglycerol |
| 3 monoacylglycerol | 12 tricapin |
| 4 monoacylglycerol | 13 diacylglycerol |
| 5 monoacylglycerol | 14 diacylglycerol |
| 6 monoacylglycerol | 15 triacylglycerol |
| 7 monoacylglycerol | 16 triacylglycerol |
| 8 monoacylglycerol | 17 diacylglycerol |
| 9 monoacylglycerol | |

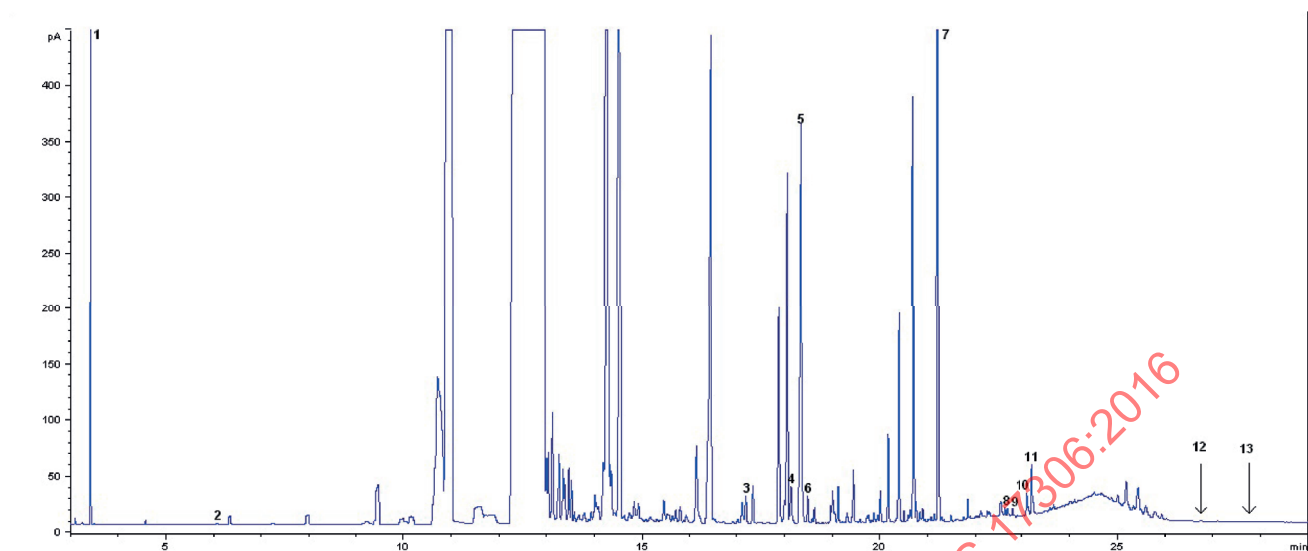
Figure A.4 — Expanded chromatogram of coconut biodiesel



Key

- | | |
|--------------------|--------------------|
| 1 ethylene glycol | 10 diacylglycerol |
| 2 glycerol | 11 diacylglycerol |
| 3 monoacylglycerol | 12 diacylglycerol |
| 4 monoacylglycerol | 13 triacylglycerol |
| 5 monoacylglycerol | 14 triacylglycerol |
| 6 monoacylglycerol | 15 triacylglycerol |
| 7 monoacylglycerol | |
| 8 tricapin | |

Figure A.5 — Expanded chromatogram of palm biodiesel



Key

- | | | | |
|---|------------------|----|-----------------|
| 1 | ethylene glycol | 8 | diacylglycerol |
| 2 | glycerol | 9 | diacylglycerol |
| 3 | monoacylglycerol | 10 | diacylglycerol |
| 4 | monoacylglycerol | 11 | diacylglycerol |
| 5 | monoacylglycerol | 12 | triacylglycerol |
| 6 | monoacylglycerol | 13 | triacylglycerol |
| 7 | tricapin | | |

Figure A.6 — Expanded chromatogram of rapeseed biodiesel

Annex B (normative)

Calculation of the coefficients for determination of the combined glycerol

As presented in ASTM D6584^[2] and in EN 14105,^[3] for biodiesel obtained from soy (*Glycine max*), canola (*Brassica napus*), cotton (*Gossypium spp.*), sunflower (*Helianthus annuus*), rapeseed (*Zea mays*) oils and their mixtures with 30 % (m/m) (maximum) of castor oil (*Ricinus communis*), the following coefficients shall be used in Formula (6) (see 10.2):

$$G_{lm} = 0,259\ 1$$

$$G_{ld} = 0,148\ 8$$

$$G_{lt} = 0,104\ 4$$

For other raw materials, Formula (B.1) shall be used.

$$G_{li} = \frac{MM_{\text{glycerol}}}{\sum MM_i \cdot v_j} \quad (\text{B.1})$$

where

G_{li} are the coefficients G_{lm} , G_{ld} and G_{lt} to be used for calculation of the bonded glycerol in Formula (6);

MM_{glycerol} is the molar mass of the glycerin, which corresponds to 92,09 kg/kmol;

MM_i is the molar mass of the mono-, di- or triacylglycerols (Table B.1), expressed in kg/kmol;

v_j is the relative contribution of the mono-, di- or triacylglycerols, expressed in % (m/m).

The following calculated coefficients for other vegetable oils can be listed:

- 1) Castor oil (*Ricinus communis*): $G_{lm} = 0,248$, $G_{ld} = 0,141\ 6$ and $G_{lt} = 0,099\ 1$;
- 2) Coconut oil (*Cocos nucifera*): $G_{lm} = 0,317\ 4$, $G_{ld} = 0,188\ 6$ and $G_{lt} = 0,134\ 2$;
- 3) Babassu oil (*Attalea speciosa Martius syn Orbignya spp.*): $G_{lm} = 0,310\ 8$, $G_{ld} = 0,184\ 0$ and $G_{lt} = 0,130\ 7$;
- 4) 4 – Palm oil (*Elaeis guineensis Dura*): $G_{lm} = 0,270\ 1$, $G_{ld} = 0,156\ 1$ and $G_{lt} = 0,109\ 8$;
- 5) 5 – Palmiste or “palm kernel” (*Elaeis oleifera*): $G_{lm} = 0,293\ 8$, $G_{ld} = 0,172\ 2$ and $G_{lt} = 0,121\ 8$