TECHNICAL SPECIFICATION

ISO/TS 17306

First edition
2016-02-01

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

Produits pétroliers — Biogazole — Détermination de glycerine libre et totale et des mono-, di- et tracylglycerols avec chromatographie gazeuse

Reference number
ISO/TS 17306:2016(E)

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Foreword

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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.

Petroleum products — Biodiesel — Determination of free and total glycerin and mono-, di- and tracylglycerols 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-trimethylsilyltrifluoro-acetamide (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 tricaprin 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) (CAS[1)] 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 Nº 56-81-5), 99,5 % minimum purity.

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

6.8 Tricaprin (CAS Nº 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 ($m_{compound} + m_{pyridine}$), 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 tricaprin (µl)	100	100	100	100	100