
**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*



Reference number
ISO/TS 17306:2016(E)

AVA Documents
Document Sharing Hub
© ISO 2016



COPYRIGHT PROTECTED DOCUMENT

© ISO 2016, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
5 Apparatus	2
6 Reagents and materials	3
7 Preparation of the apparatus	3
8 Sampling	4
9 Preparation of the standard solutions	4
9.1 Preparation of the stock solutions.....	4
9.2 Preparation of the calibration curve.....	5
9.3 Preparation of the sample.....	6
9.4 Procedure.....	7
10 Calculation and expression of results	8
11 Precision	10
11.1 General.....	10
11.2 Repeatability, r	10
11.3 Reproducibility, R	10
12 Test report	11
Annex A (normative) Check chromatograms	12
Annex B (normative) Calculation of the coefficients for determination of the combined glycerol	16
Annex C (informative) Information on interlaboratory study	20
Bibliography	21

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*.

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 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.