SLOVENSKI STANDARD
SIST EN 16934:2017

01-oktober-2017

Goriva za motorna vozila ter maščobni in oljni derivati - Določevanje sterilglikozidov v metilnih estrih maščobnih kislin (FAME) - Metoda z GC-MS s predhodnim čiščenjem s SPE

Automotive fuels and fat and oil derivates - Determination of steryl glycosides in fatty acid methyl esters (FAME) - Method by GC-MS with prior purification by SPE

Kraftstoffe und Erzeugnisse aus Fetten und Ölen - Bestimmung des Gehaltes an Sterylglycosiden in Fettsäure-Methylester (FAME) - Verfahren mittels GC-MS und vorausgehender Reinigung mit SPE

Carburants pour automobiles et produits dérivés des corps gras - Détermination des stérols glucosides dans les esters méthyliques d'acides gras (EMAG) - Méthode par GC-MS avec purification préalable par SPE

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN 16934:2017) has been prepared by Technical Committee CEN/TC 19 “Gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2018, and conflicting national standards shall be withdrawn at the latest by February 2018.

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1 Scope

This European Standard describes a procedure for the determination of steryl glycosides (SG) content in fatty acid methyl esters (FAME) in a range between 20 mg/kg and 38 mg/kg.

NOTE Steryl glycosides (SG) are mainly present in vegetable oils.

WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of users of this standard to take appropriate measures to ensure the safety and health of personnel prior to application of the standard, and fulfil statutory and regulatory requirements for this purpose.

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.

EN ISO 3170, *Petroleum liquids - Manual sampling (ISO 3170)*

EN ISO 3171, *Petroleum liquids - Automatic pipeline sampling (ISO 3171)*

3 Principle

The steryl glycosides (SG) content of a FAME sample is determined by gas chromatography with mass spectrometry detector (GC-MS) with cold on column (COC) or programmed temperature vaporization (PTV) injection and measuring via selected ion monitoring (SIM).

Before injection into the GC instrument the sample is purified via solid phase extraction (SPE) and derivatized with BSA+TMCS (4.2).

The SG content is quantified by using cholesteryl-β-D-glucopyranoside (4.3) as internal standard (IS).

4 Reagents and materials

Use only reagents of recognized analytical grade, unless otherwise specified.

4.1 Pyridine, max. 0,1 % water.

4.2 N,O-bis(trimethylsilyl)acetamide + 5 % trimethylchlorosilane (BSA+TMCS) silylation reagent.

4.3 Cholesteryl-β-D-glucopyranoside (CAS number: 7073-61-2) [1)], which purity has been verified.

4.4 Methyl tert butyl ether (MTBE).

4.5 n-Heptane.

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1) Cholesteryl-β-D-glucopyranoside (10 mg, PR463-XX-S10) provided by ASG Analytik-Service Gesellschaft mbH, Cholesterol β-D-glucoside (28609-10MG) provided by Sigma-Aldrich, mixed SG standard, 98 % (soybean), provided by Larodan (5 mg, ref. no. 60-1020-4) or Matreya (25 mg, ref. no. 1117) have been found suitable for use. Equivalent products may be used if they can be shown to lead to the same results. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

4.6 Acetone.

4.7 Methanol.

4.8 n-Heptane/MTBE 2:1 (*V*/*V*) mixture.

4.9 Toluene.

4.10 Internal standard solution of 100 mg/l Cholesteryl-β-D-glucopyranoside. Dissolve approximately 10 mg of cholesteryl-β-D-glucopyranoside (4.3) (accuracy ± 0,1 mg) in a 100 ml calibrated flask with 10 ml of pyridine (4.1). Fill up to the mark using toluene (4.9) as solvent. Calculate the exact concentration in mg/ml (approximately 0,1 mg/ml). Alternatively, a commercially available IS solution may be used.

4.11 Carrier gas, Helium.

5 Apparatus

Usual laboratory equipment and, in particular, the following:

5.1 Gas chromatograph, equipped with a cold on-column (COC) or programmed temperature vaporization (PTV) injector, a temperature programmable oven and a mass spectrometry detector.

5.2 Capillary column, high temperature (HT) capable for which the following characteristics are suggested:

— 95 % dimethyl-5 % diphenyl polysiloxane stationary phase,

— length 15 m,

— internal diameter 250 µm,

— film thickness 0,1 µm.

5.3 Operating conditions:

— Injection Volume: 1 µl,

— column temperature program: 100 °C hold for 2 min, programmed at 10 °C/min up to 370 °C, final temperature hold for 6 min,

— carrier gas (Helium) constant flow: 1,2 ml/min,

— temperature MS Source: minimum 300 °C,

— temperature MS Quad: minimum 180 °C,

— solvent delay: 20 min, under above mentioned conditions,

— SIM Mode: The analysis is run in SIM mode, the following ions are used as sum for both analytes and IS: 147, 204, 217 m/z,

— recommended temperature of MS transfer line: minimum 370 °C.

5.4 Analytical balance, accuracy ± 0,1 mg.

5.5 Precision pipette, 200 µl capacity.

5.6 Precision pipette, 1 ml capacity.

5.7 SPE cartridge[2)], Silica gel, with no bonded phase, 1 g.

5.8 Volumetric flask, 100 ml capacity.

5.9 Graduated cylinder, 10 ml capacity.

5.10 Heating block, capable of keeping and maintaining the temperature of 80 °C with an accuracy of ± 2 °C.

5.11 Test tubes, 10 ml capacity, with plastic stoppers or screw caps.

6 Sampling

Unless otherwise specified, obtain samples in accordance with the procedures given in EN ISO 3170 or EN ISO 3171.

7 Procedure

7.1 Sample purification

7.1.1 Weigh 5,0 ± 0,1 g of sample into a suitable bottle with accuracy of 0,01 g.

7.1.2 Add 500 µl of pyridine (4.1) using a precision pipette (5.6).

7.1.3 Add 1 ml of the internal standard solution (4.10) using a precision pipette (5.6).

7.1.4 Add 5 ml of n-heptane/MTBE (2:1 *V*/*V*) mixture (4.8) using a graduated cylinder (5.9).

7.1.5 Close the bottle and thoroughly shake the mixture.

All elutions on the SPE cartridge shall be done using gravity elution only, do not apply vacuum.

7.1.6 Condition the SPE cartridge (5.7) by rinsing with 4 ml of n-heptane (4.5).

7.1.7 Gradually transfer all the sample mixture (7.1.5) onto the SPE cartridge.

7.1.8 When the entire sample has been transferred onto the cartridge rinse it with 5 ml of n-heptane/MTBE (2:1 *V*/*V*) mixture.

7.1.9 Discard all collected eluent.

7.1.10 Elute the steryl glycosides by rinsing the SPE cartridge with 4 ml of acetone (4.6) followed by 4 ml of methanol (4.7) and collect the eluent in a 10 ml test tube (5.11).

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2) SPE-Cartridges brand "Varian HF Mega BE-SI 1GM 6 ml (Bond Elute) order number 14256008 or Phenomenex STRATA-SI (70 A) order number 8B-S012-JCH" have been found suitable for use. Equivalent products may be used if they can be shown to lead to the same results. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

7.1.11 Evaporate the eluent by heating to 60 °C using the heating block (5.10) and applying a gentle flow of dry nitrogen until completely dry.

7.2 Sample preparation and analysis

7.2.1 Take the purified sample and add approximately 200 µl of pyridine (4.1) and approximately 200 µl of silylating reagent mixture (4.2).

7.2.2 Close the test tube, shake thoroughly and let the sample react in the heating block at 80 °C, whilst shaking every 15 min.

7.2.3 Take the sample from the heating block and allow the sample to cool to room temperature.

7.2.4 Open the test tube, add 1 ml of heptane, carefully close again and mix thoroughly.

7.2.5 Inject 1 µl of this solution into the GC and start the GC and data collection system.

7.3 Identification and integration peaks

The identification of IS as well as SG peaks is carried out by comparing the retention times of known compounds. It is therefore advised, in order to identify the IS and the SG peaks, to inject the pure compound and an isolated sample of SG from commercial FAME.

Samples are subject to derivatization in any case.

Typical chromatograms with internal standard and steryl glycoside peaks are given in Annex A.

Record the area of the IS peak and the area of the SG mixture peak.

8 Calculation of results

The SG content is calculated using a relative response factor of 1,00.

Calculate the concentration of steryl glycosides in the sample using the formula:

$$SG = \left(\frac{A_{SG}}{A_{IS}}\right) \times \left(\frac{C_{IS} \times V_{IS}}{m_S}\right) \times 1\,000 \qquad (1)$$

where

SG is the concentration of steryl glycosides (mg/kg);

A_{IS} is the peak area of internal standard cholesteryl-β-D-glucopyranoside;

A_{SG} is the sum of the peak areas of the individual steryl glycosides;

C_{IS} is the concentration of the IS solution in mg/ml (approximately 0,1 mg/ml, use the correctly calculated value);

V_{IS} is the volume of IS added, in ml;

m_S is the mass of the FAME sample, in g.